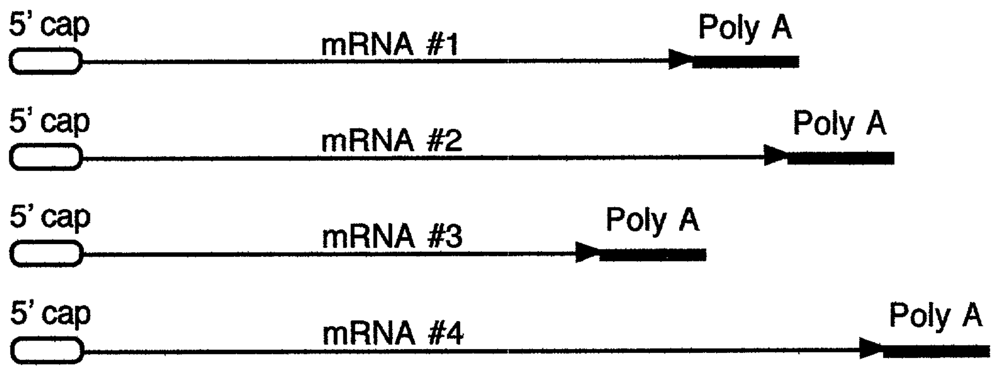
Library of mRNA analytes



Library of mRNA analytes bound to an array

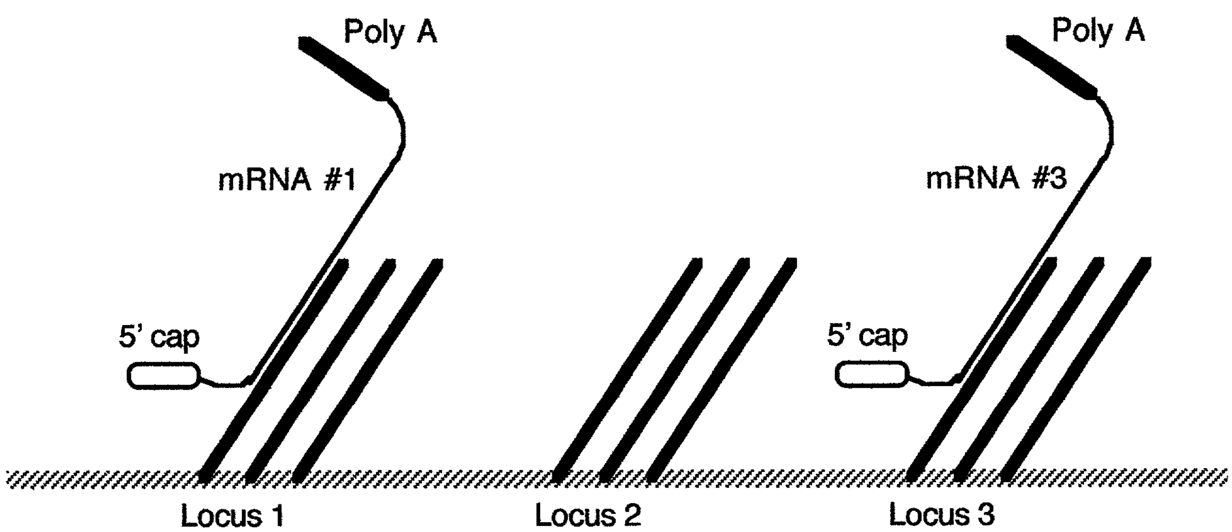


Figure 1

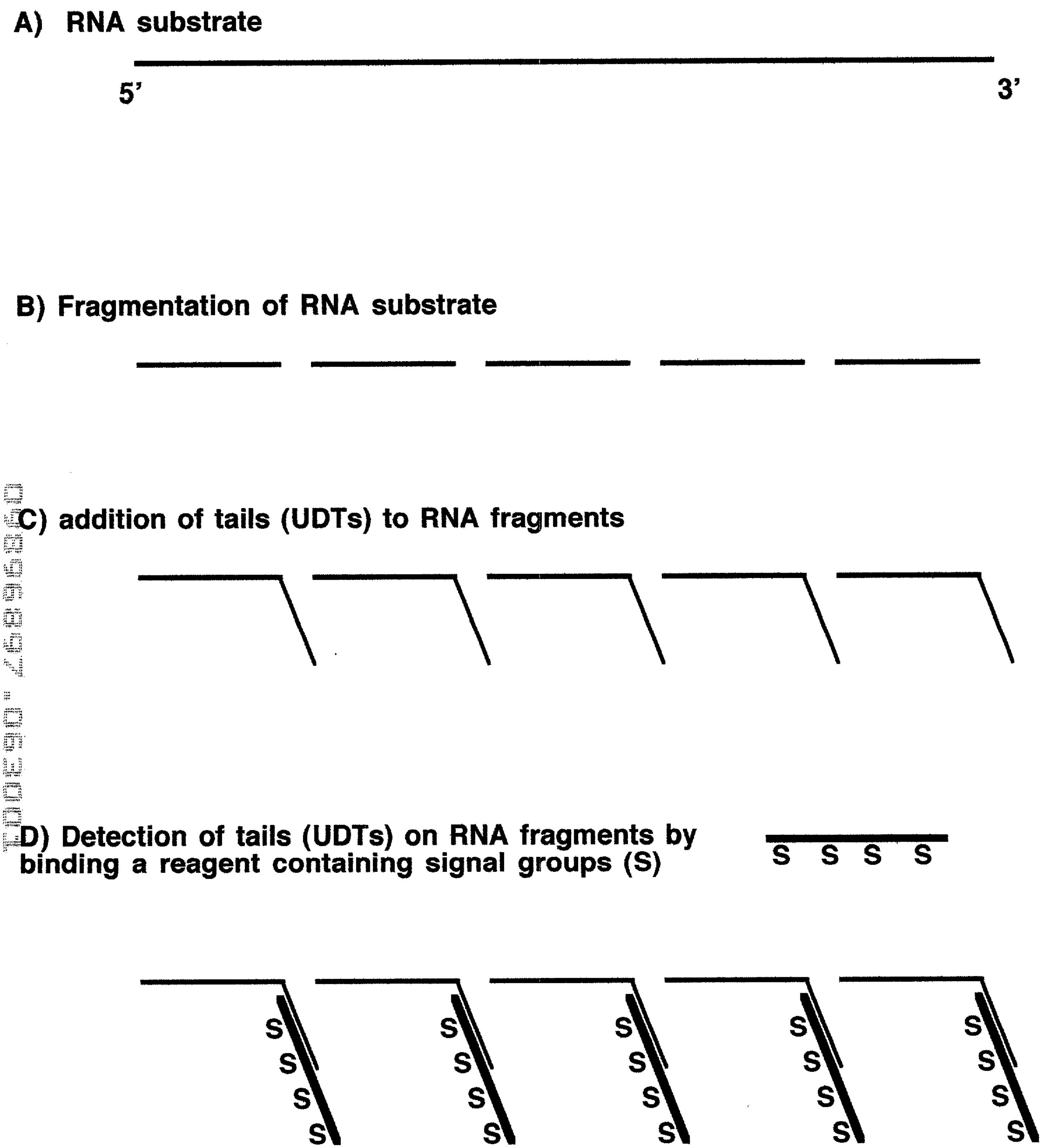

A) RNA substrate
5'
3'
B) Fragmentation of RNA substrate
C) addition of tails (UDTs) to RNA fragments
D) Detection of tails (UDTs) on RNA fragments by binding a reagent containing signal groups (S)
S S S S
S S S S
S S S S
S S S S
S S S S
S S S S

FIGURE 2

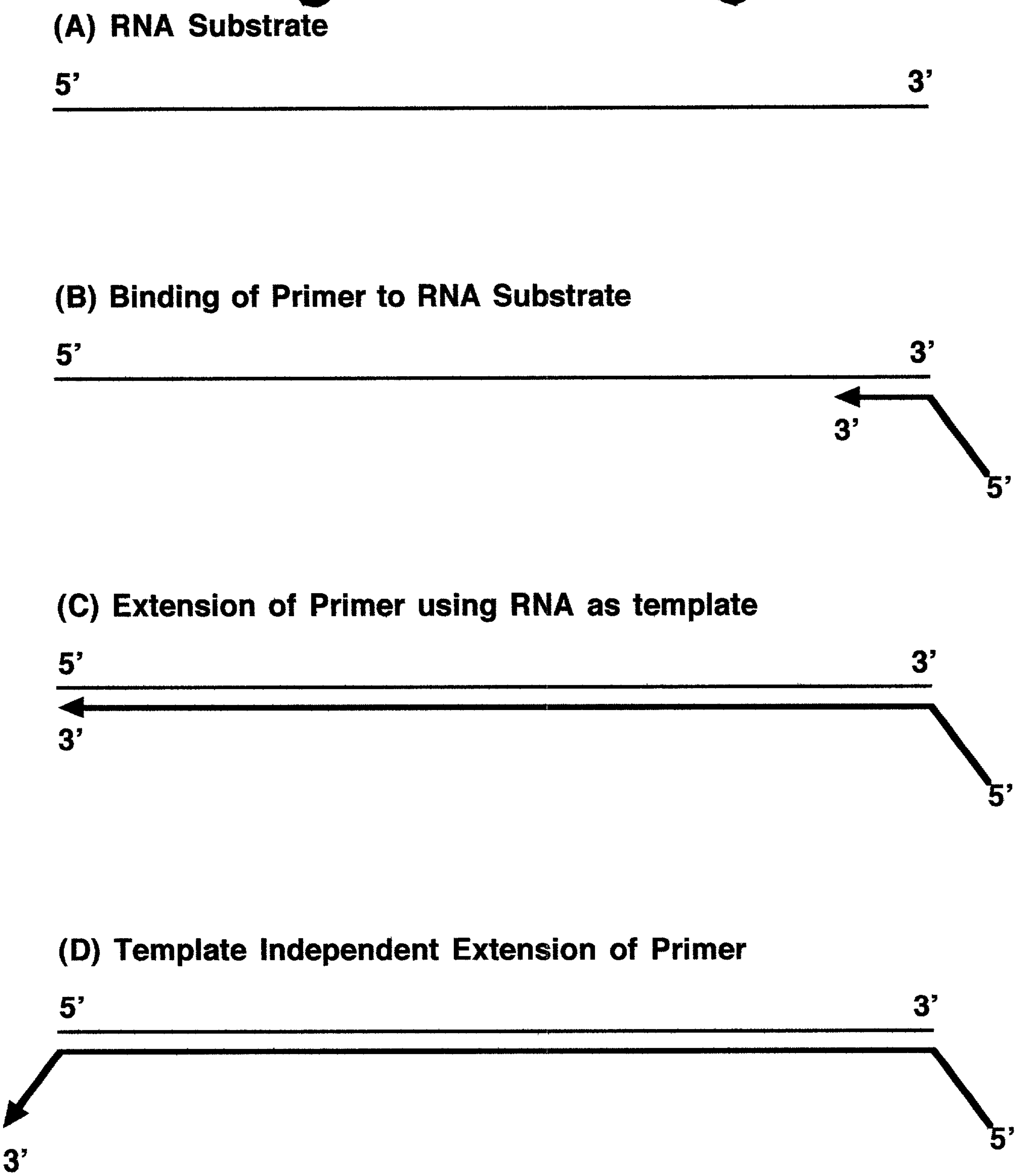

(A) RNA Substrate
5'
3'
(B) Binding of Primer to RNA Substrate
5'
3'
3'
5'
(C) Extension of Primer using RNA as template
5'
3'
3'
5'
(D) Template Independent Extension of Primer
5'
3'
5'
3'

FIGURE 3

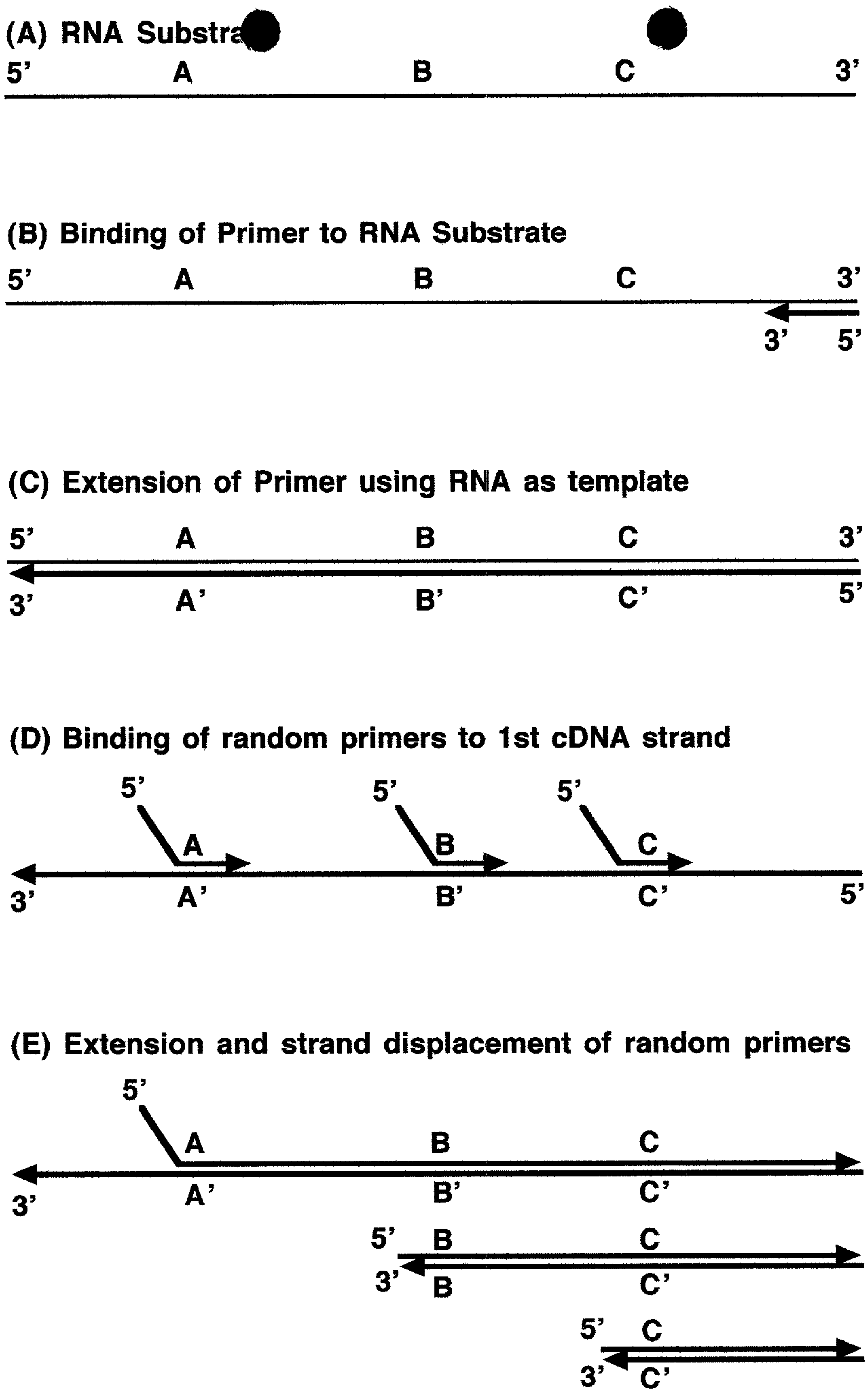
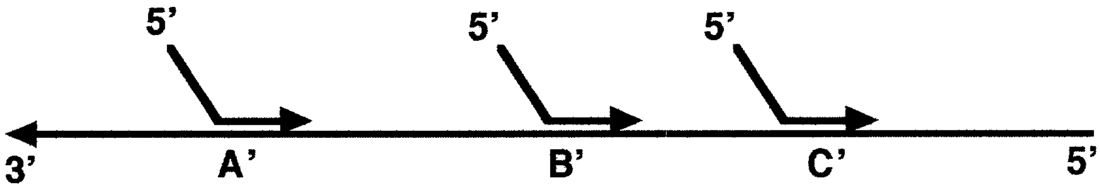
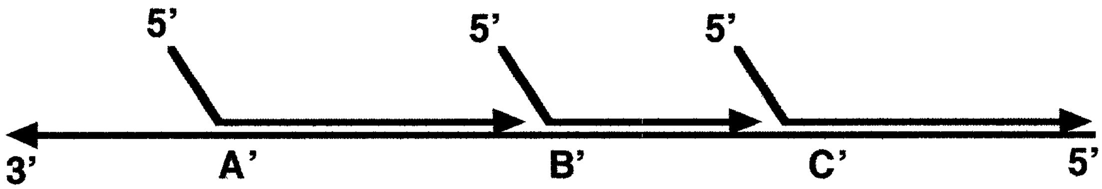

(A) RNA Substra
5' A B C 3'
(B) Binding of Primer to RNA Substrate
5' A B C 3'
3' 5'
(C) Extension of Primer using RNA as template
5' A B C 3'
3' A' B' C' 5'
(D) Binding of random primers to 1st cDNA strand
5' 5' 5'
A B C
3' A' B' C' 5'
(E) Extension and strand displacement of random primers
5'
A B C
3' A' B' C'
5' B C
3' B C'
5' C
3' C'

FIGURE 4

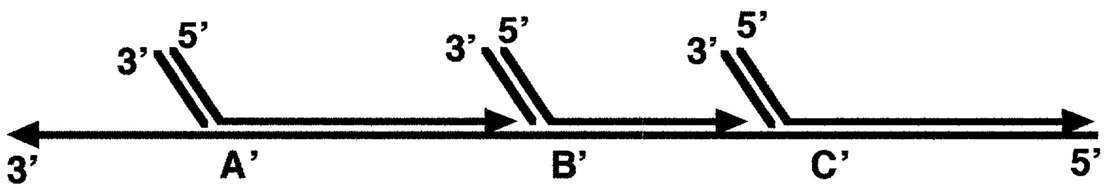
(1) Binding of random primers to 1st cDNA strand



(2) Extension of random primers using 1st cDNA strand as template



(3-a) Creation of functional promoters by binding of complementary strand



(3-a) Creation of functional promoters by self-complementary sequences

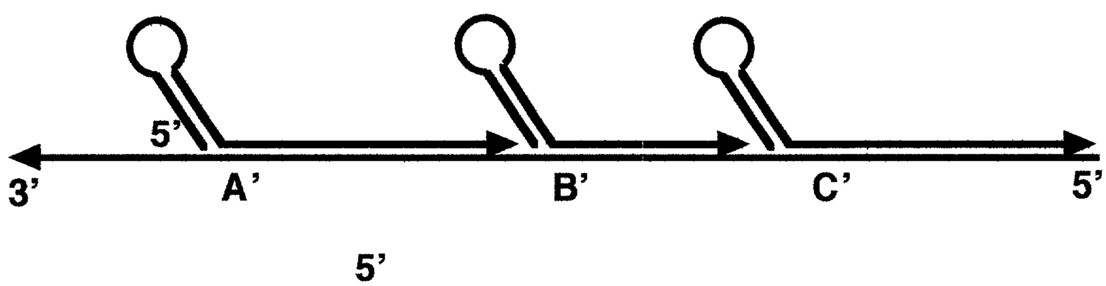


FIGURE 5

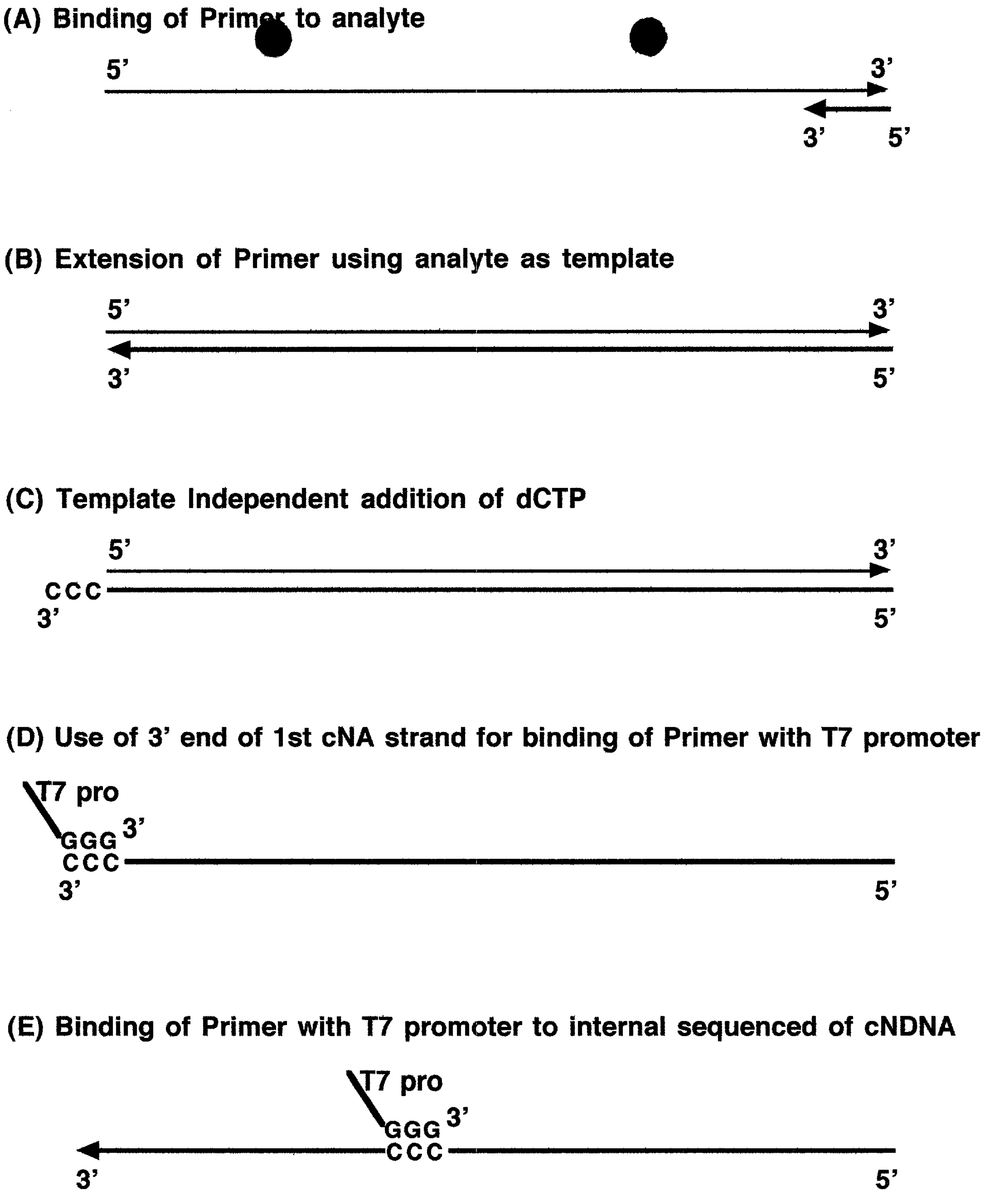

(A) Binding of Primer to analyte
5'
3'
3'
5'
(B) Extension of Primer using analyte as template
5'
3'
3'
5'
(C) Template Independent addition of dCTP
5'
3'
CCC
3'
5'
(D) Use of 3' end of 1st cNA strand for binding of Primer with T7 promoter
T7 pro
GGG
3'
CCC
3'
5'
(E) Binding of Primer with T7 promoter to internal sequenced of cNDNA
T7 pro
GGG
3'
CCC
3'
5'

FIGURE 6

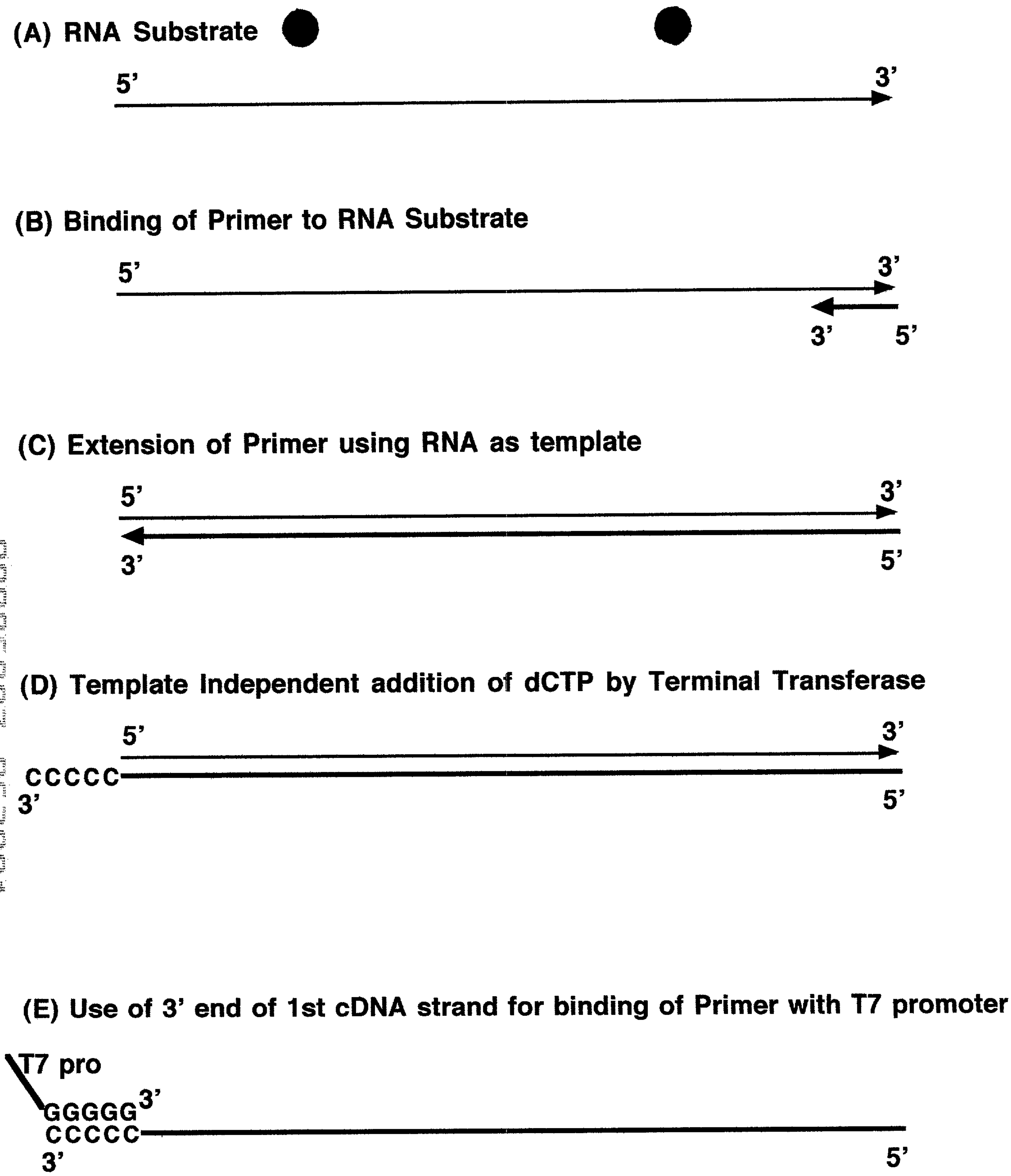

(A) RNA Substrate
5'
3'
(B) Binding of Primer to RNA Substrate
5'
3'
3'
5'
(C) Extension of Primer using RNA as template
5'
3'
3'
5'
(D) Template Independent addition of dCTP by Terminal Transferase
5'
3'
CCCCC
3'
5'
(E) Use of 3' end of 1st cDNA strand for binding of Primer with T7 promoter
T7 pro
GGGGG
3'
CCCCC
3'
5'

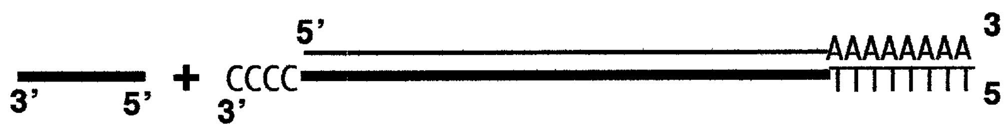
FIGURE 7

1) analyte

2) cNA copy made from analyte

3a) double-stranded oligonucleotide ligated to RNA/DNA hybrid by T4 DNA ligase

3b) single-stranded oligonucleotide ligated to a single-stranded 3' tail by T4 RNA ligase



3c) double-stranded oligonucleotide ligated to single-stranded 3' tail by T4 DNA ligase

FIGURE 8

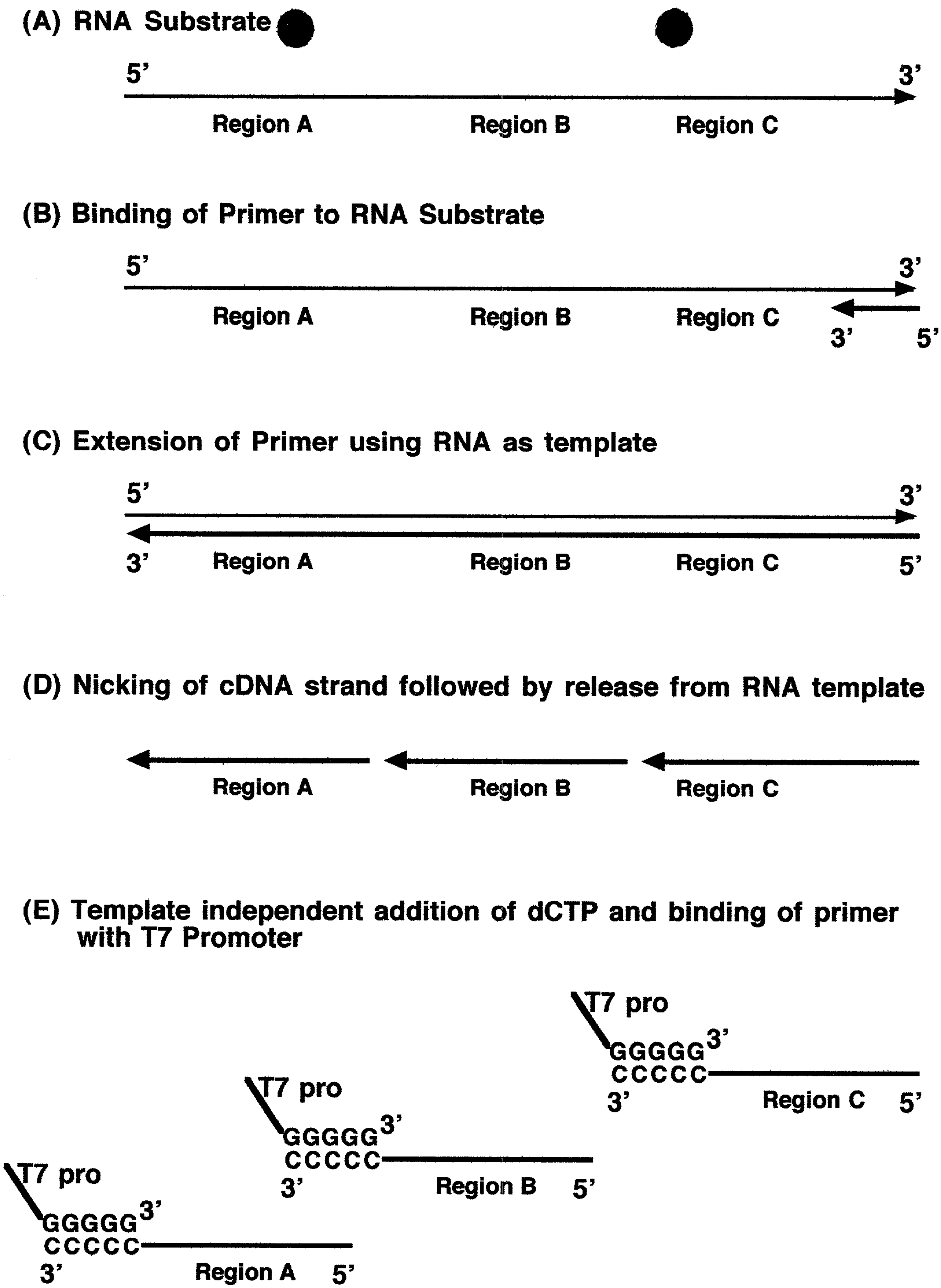
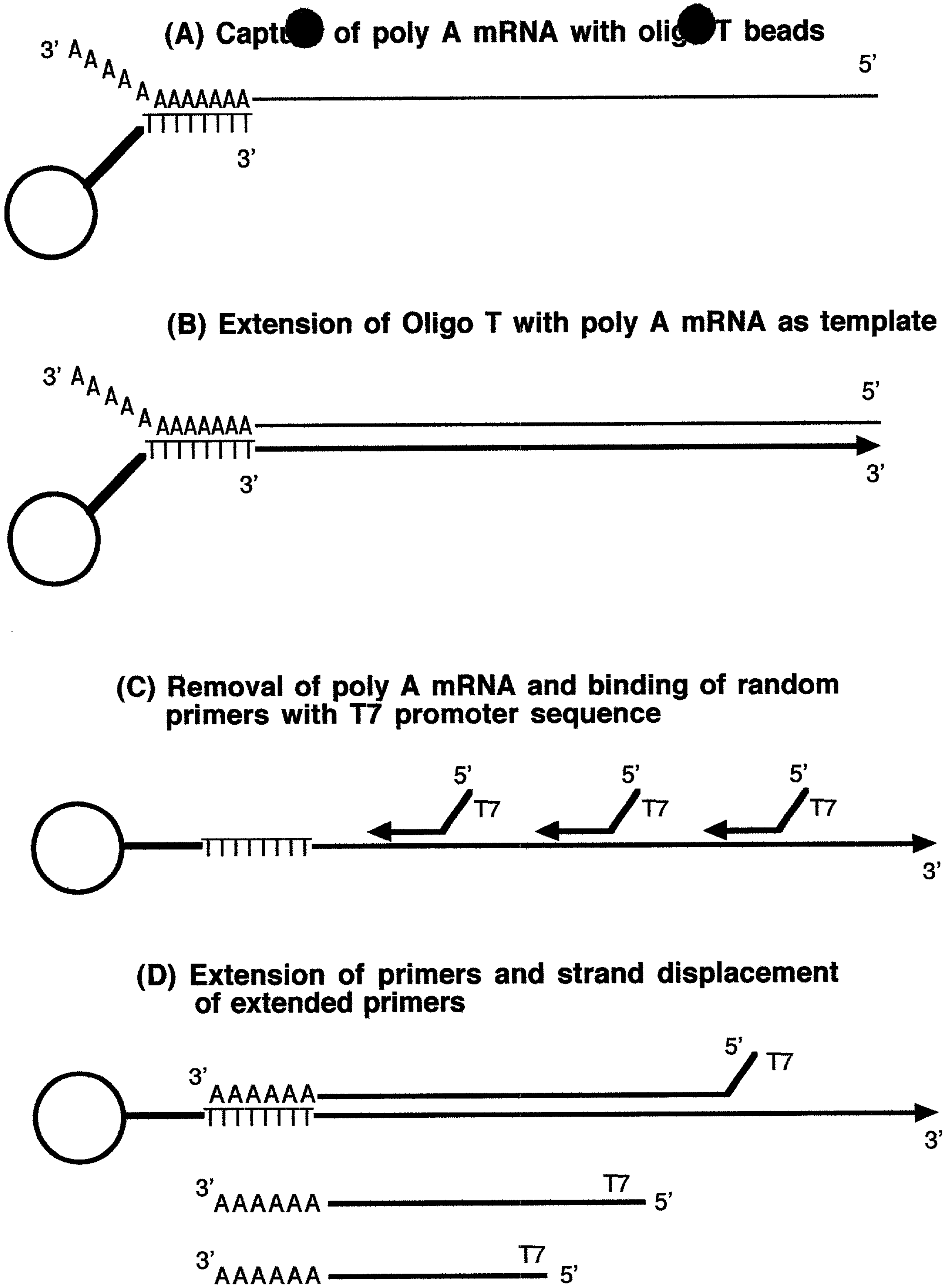

(A) RNA Substrate
5'
3'
Region A
Region B
Region C
(B) Binding of Primer to RNA Substrate
5'
3'
Region A
Region B
Region C
3'
5'
(C) Extension of Primer using RNA as template
5'
3'
3'
Region A
Region B
Region C
5'
(D) Nicking of cDNA strand followed by release from RNA template
Region A
Region B
Region C
(E) Template independent addition of dCTP and binding of primer with T7 Promoter
T7 pro
GGGGG
3'
CCCCC
3'
Region C
5'
T7 pro
GGGGG
3'
CCCCC
3'
Region B
5'
T7 pro
GGGGG
3'
CCCCC
3'
Region A
5'

FIGURE 9



(A) Capt of poly A mRNA with olig T beads
3' AAAAAAAAAAAA
TTTTTTTT
3'
5'
(B) Extension of Oligo T with poly A mRNA as template
3' AAAAAAAAAAAA
TTTTTTTT
3'
5'
3'
(C) Removal of poly A mRNA and binding of random primers with T7 promoter sequence
TTTTTTTT
5'
T7
5'
T7
5'
T7
3'
(D) Extension of primers and strand displacement of extended primers
3' AAAAAA
TTTTTTTT
5'
T7
3'
3' AAAAAA
T7
5'
3' AAAAAA
T7
5'

FIGURE 10

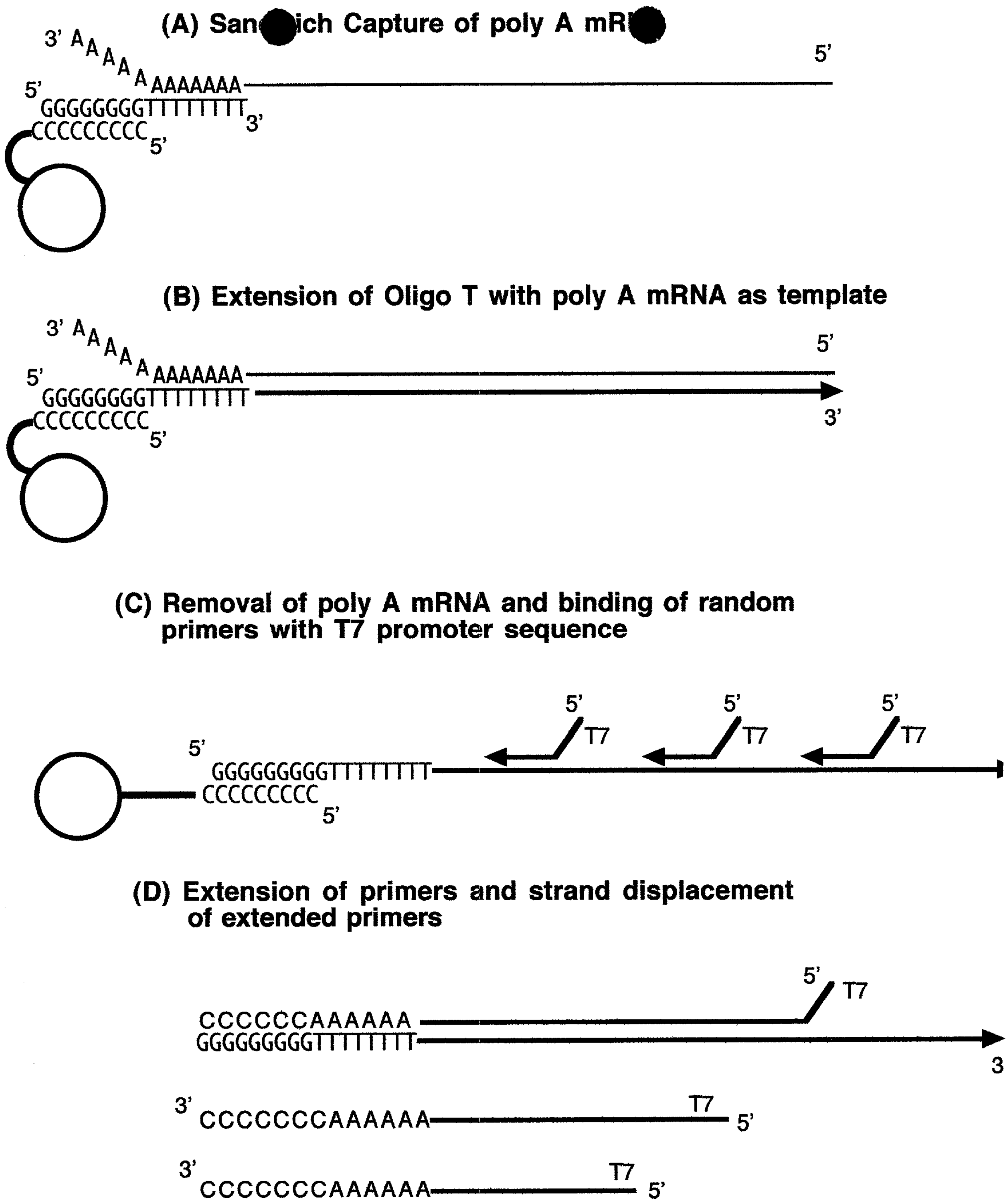

(A) San ich Capture of poly A mR
3' AAAAA AAAAAAA
5'
5'
GGGGGGGGTTTTTTTT
3'
CCCCCCCCC
5'
(B) Extension of Oligo T with poly A mRNA as template
3' AAAAA AAAAAAA
5'
5'
GGGGGGGGTTTTTTTT
3'
CCCCCCCCC
5'
(C) Removal of poly A mRNA and binding of random primers with T7 promoter sequence
5'
T7
5'
T7
5'
T7
5'
GGGGGGGGGTTTTTTTT
CCCCCCCCC
5'
(D) Extension of primers and strand displacement of extended primers
5'
T7
CCCCCCAAAAAA
GGGGGGGGGTTTTTTTT
3
3'
CCCCCCCAAAAAA
T7
5'
3'
CCCCCCCAAAAAA
T7
5'

FIGURE 11

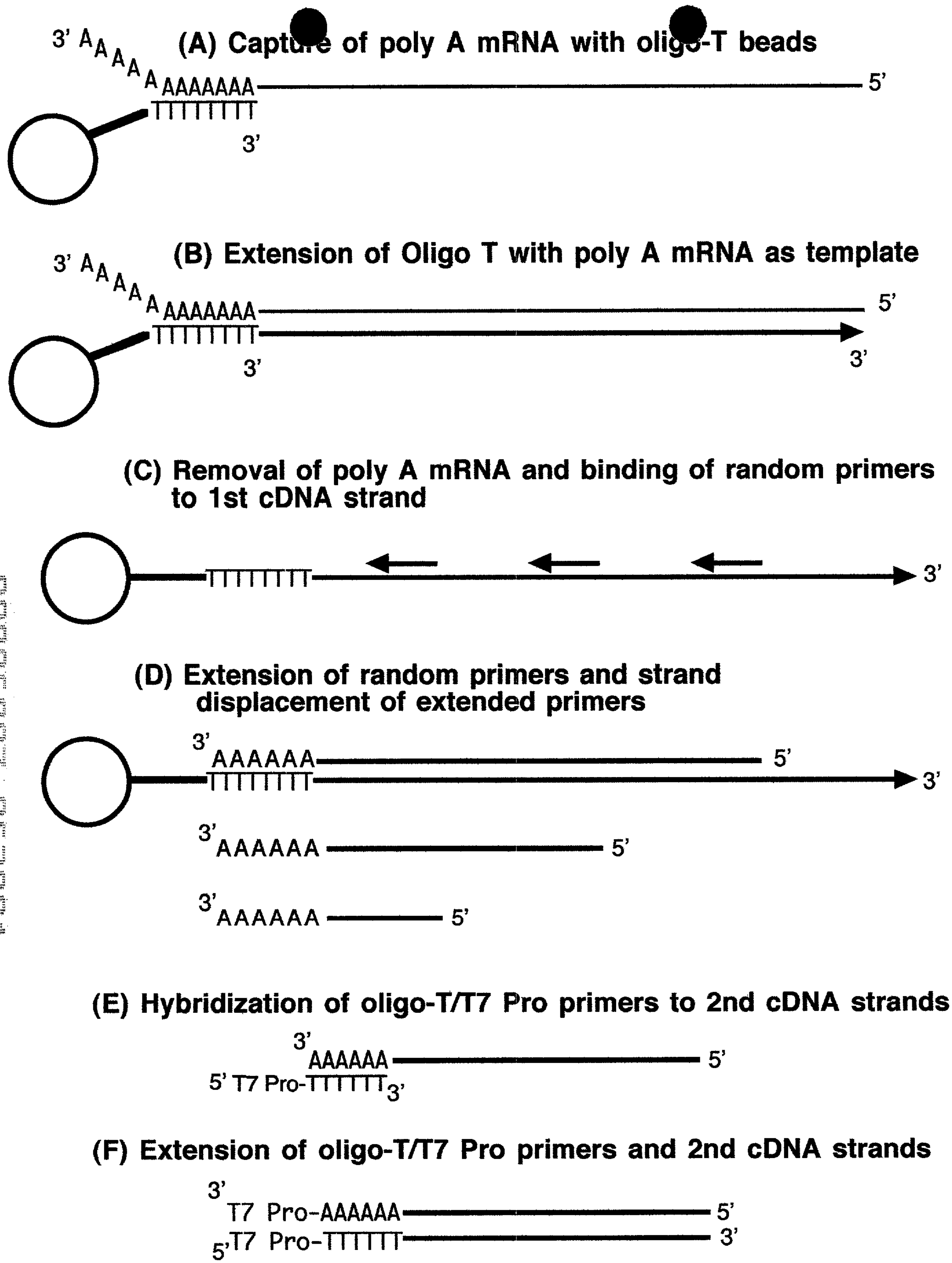

(A) Capture of poly A mRNA with oligo-T beads
3' AAAAAAAAAAAA
5'
TTTTTTTT
3'
(B) Extension of Oligo T with poly A mRNA as template
3' AAAAAAAAAAAA
5'
TTTTTTTT
3'
3'
(C) Removal of poly A mRNA and binding of random primers to 1st cDNA strand
TTTTTTTT
3'
(D) Extension of random primers and strand displacement of extended primers
3' AAAAAA
5'
TTTTTTTT
3'
3' AAAAAA
5'
3' AAAAAA
5'
(E) Hybridization of oligo-T/T7 Pro primers to 2nd cDNA strands
3' AAAAAA
5'
5' T7 Pro-TTTTTT 3'
(F) Extension of oligo-T/T7 Pro primers and 2nd cDNA strands
3' T7 Pro-AAAAAA
5'
5' T7 Pro-TTTTTT
3'

FIGURE 12

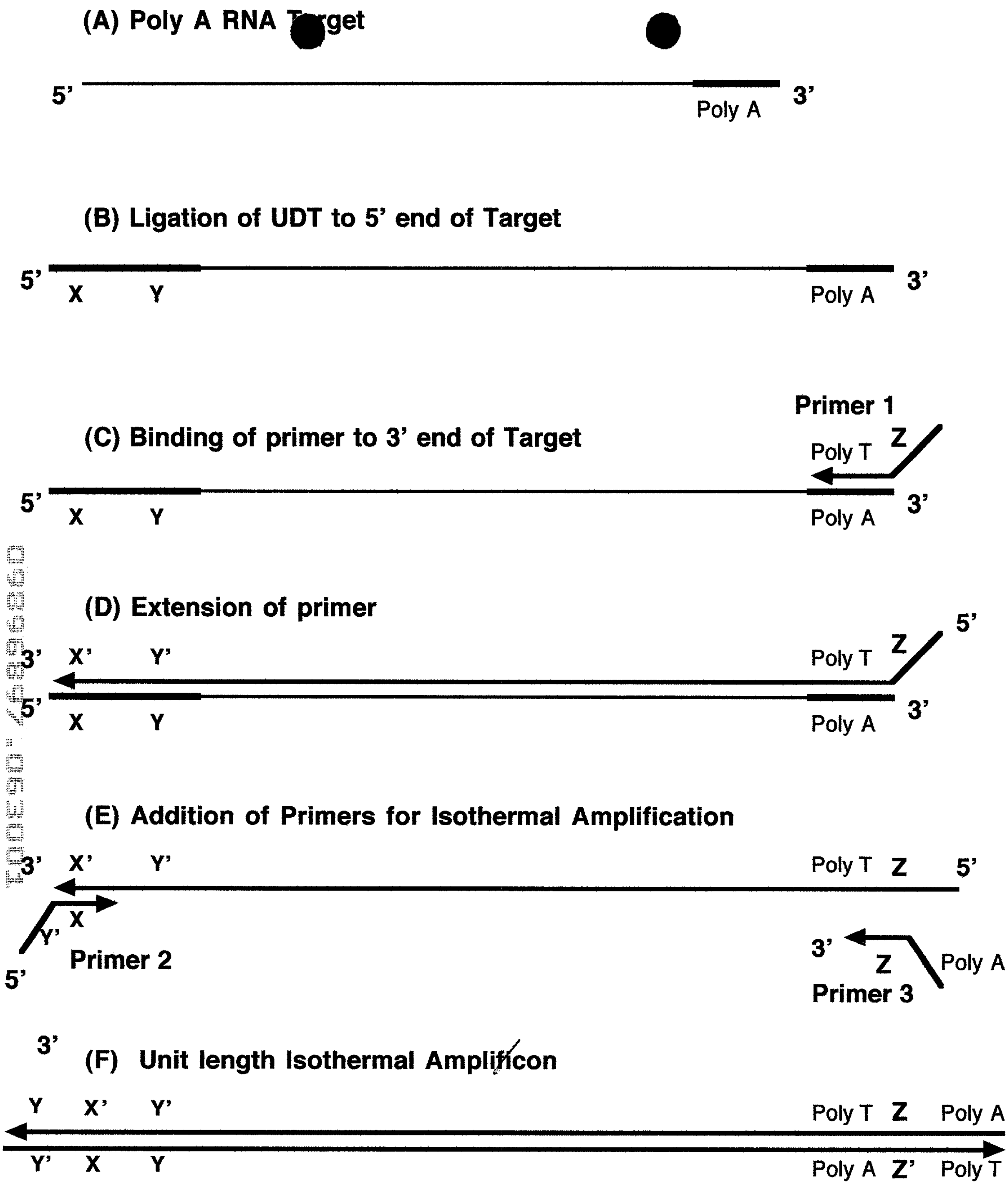

(A) Poly A RNA Target
5'
3'
Poly A
(B) Ligation of UDT to 5' end of Target
5'
X
Y
3'
Poly A
(C) Binding of primer to 3' end of Target
Primer 1
Poly T
Z
5'
X
Y
3'
Poly A
(D) Extension of primer
5'
3'
X'
Y'
Poly T
Z
5'
X
Y
3'
Poly A
(E) Addition of Primers for Isothermal Amplification
3'
X'
Y'
Poly T
Z
5'
X
Y'
Primer 2
5'
3'
Z
Poly A
Primer 3
3'
(F) Unit length Isothermal Amplificon
Y
X'
Y'
Poly T
Z
Poly A
Y'
X
Y
Poly A
Z'
Poly T

FIGURE 13

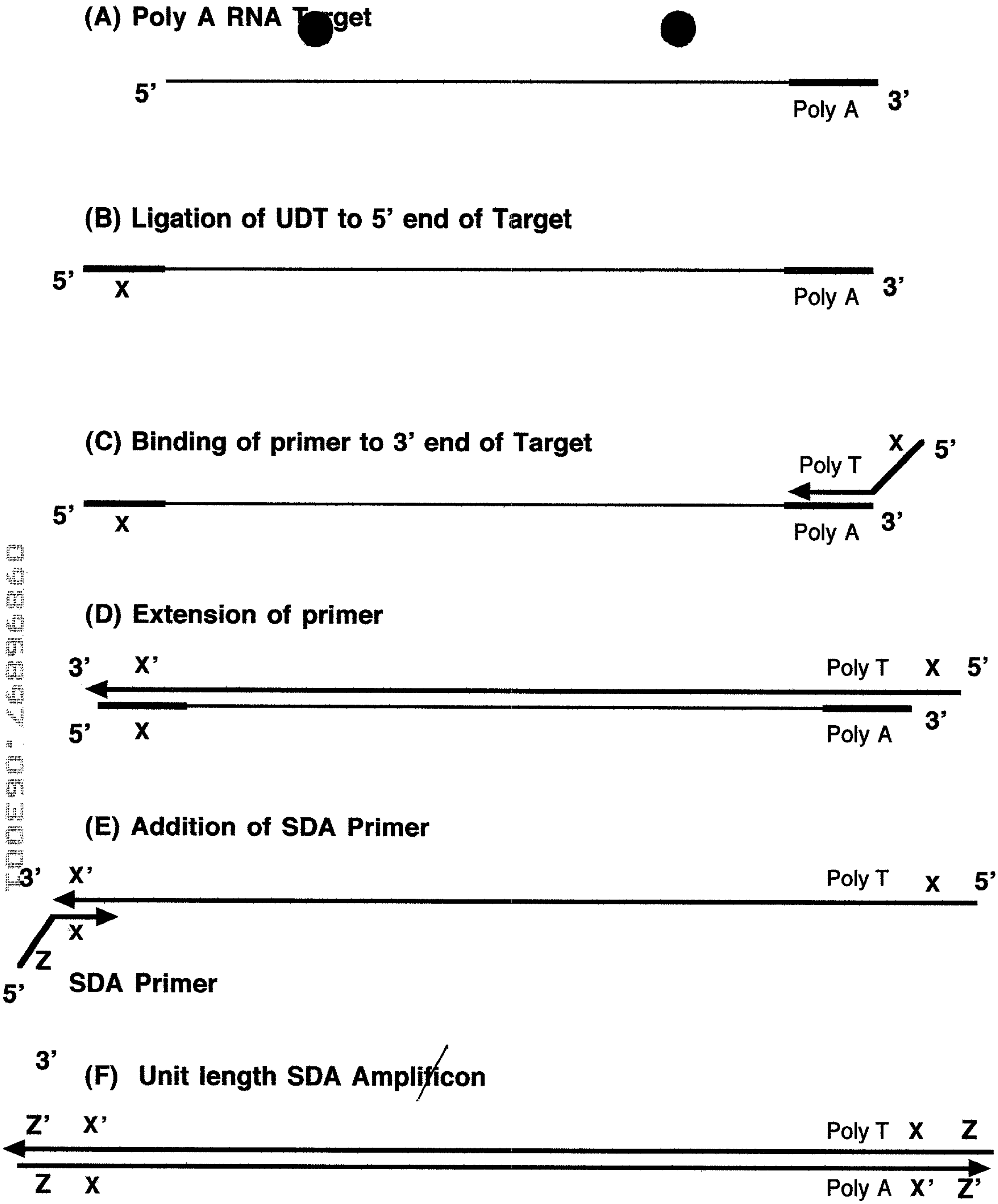

(A) Poly A RNA Target
5'
Poly A
3'
(B) Ligation of UDT to 5' end of Target
5'
X
Poly A
3'
(C) Binding of primer to 3' end of Target
Poly T
X
5'
5'
X
Poly A
3'
(D) Extension of primer
3'
X'
Poly T
X
5'
5'
X
Poly A
3'
(E) Addition of SDA Primer
3'
X'
Poly T
X
5'
X
Z
5'
SDA Primer
3'
(F) Unit length SDA Amplicon
Z'
X'
Poly T
X
Z
Z
X
Poly A
X'
Z'

FIGURE 14

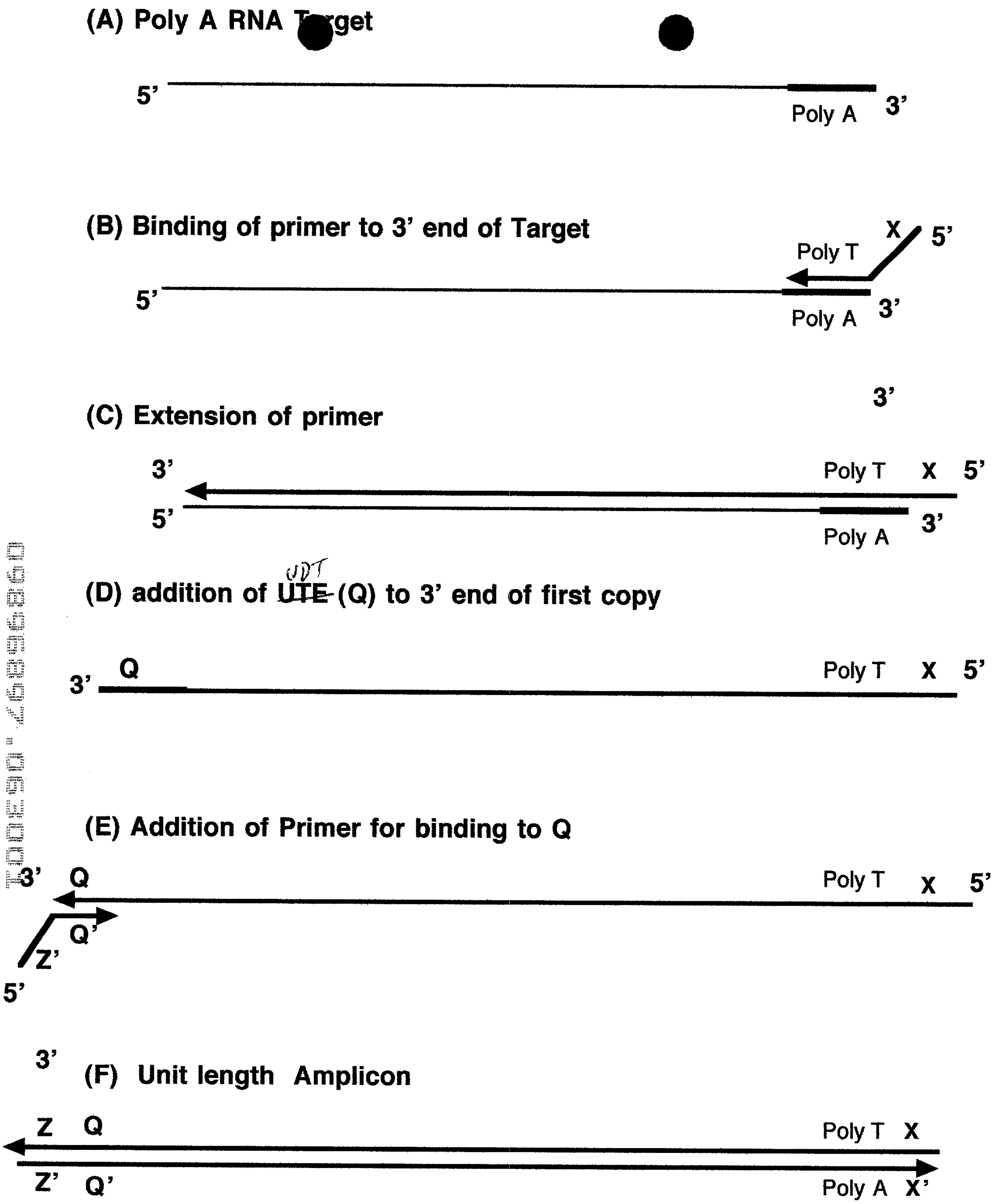
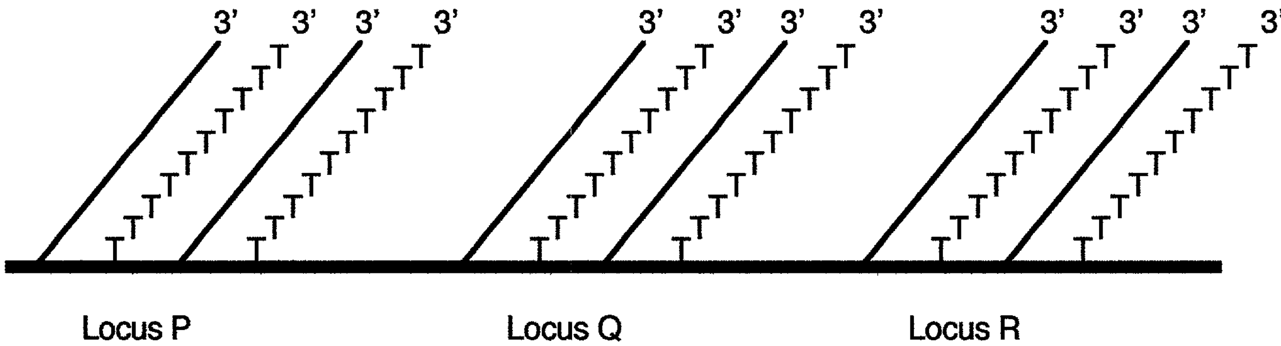

(A) Poly A RNA Target
5'
Poly A
3'
(B) Binding of primer to 3' end of Target
X
5'
Poly T
5'
Poly A
3'
(C) Extension of primer
3'
3'
Poly T
X
5'
5'
Poly A
3'
(D) addition of ~~UTE~~ UDT (Q) to 3' end of first copy
3'
Q
Poly T
X
5'
(E) Addition of Primer for binding to Q
3'
Q
Poly T
X
5'
Q'
Z'
5'
3'
(F) Unit length Amplicon
Z
Q
Poly T
X
Z'
Q'
Poly A
X'

FIGURE 15

(1) Array with SPE's complementary to analyte "P" at Locus P, SPE's complementary to analyte "Q" at Locus Q and SPE's complementary to analyte "R" at Locus R and with UPE's comprising Poly T sequences at all three loci



(2) Binding of analyte "P" to corresponding SPE at Locus P

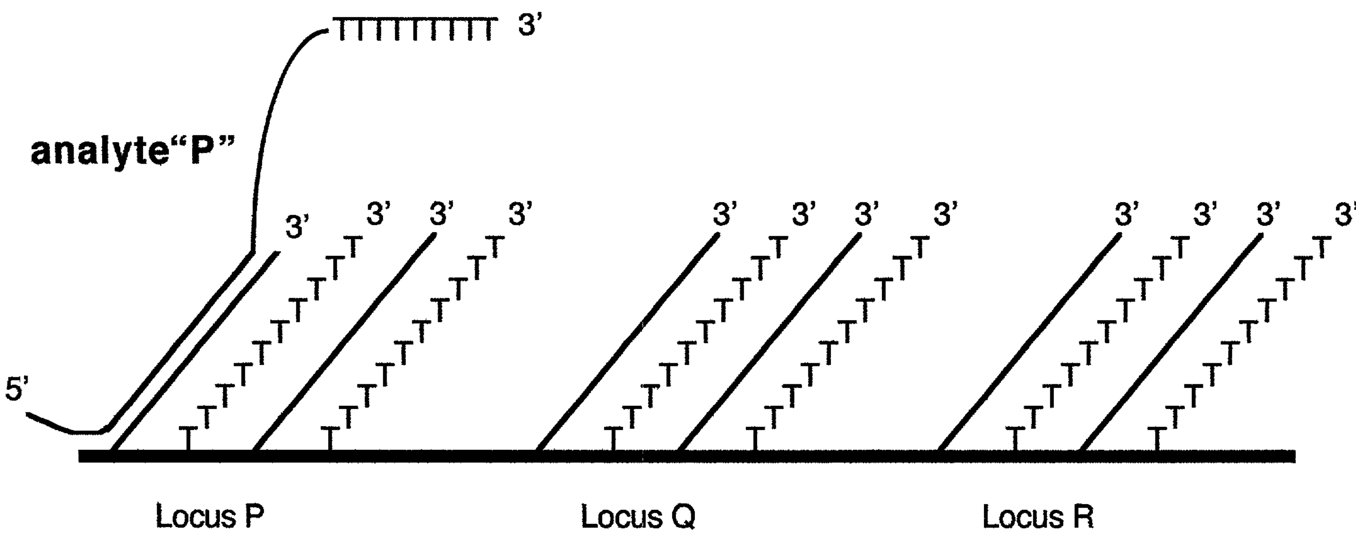


Figure 16

Binding of an analyte to an array with SPE's and UPE's

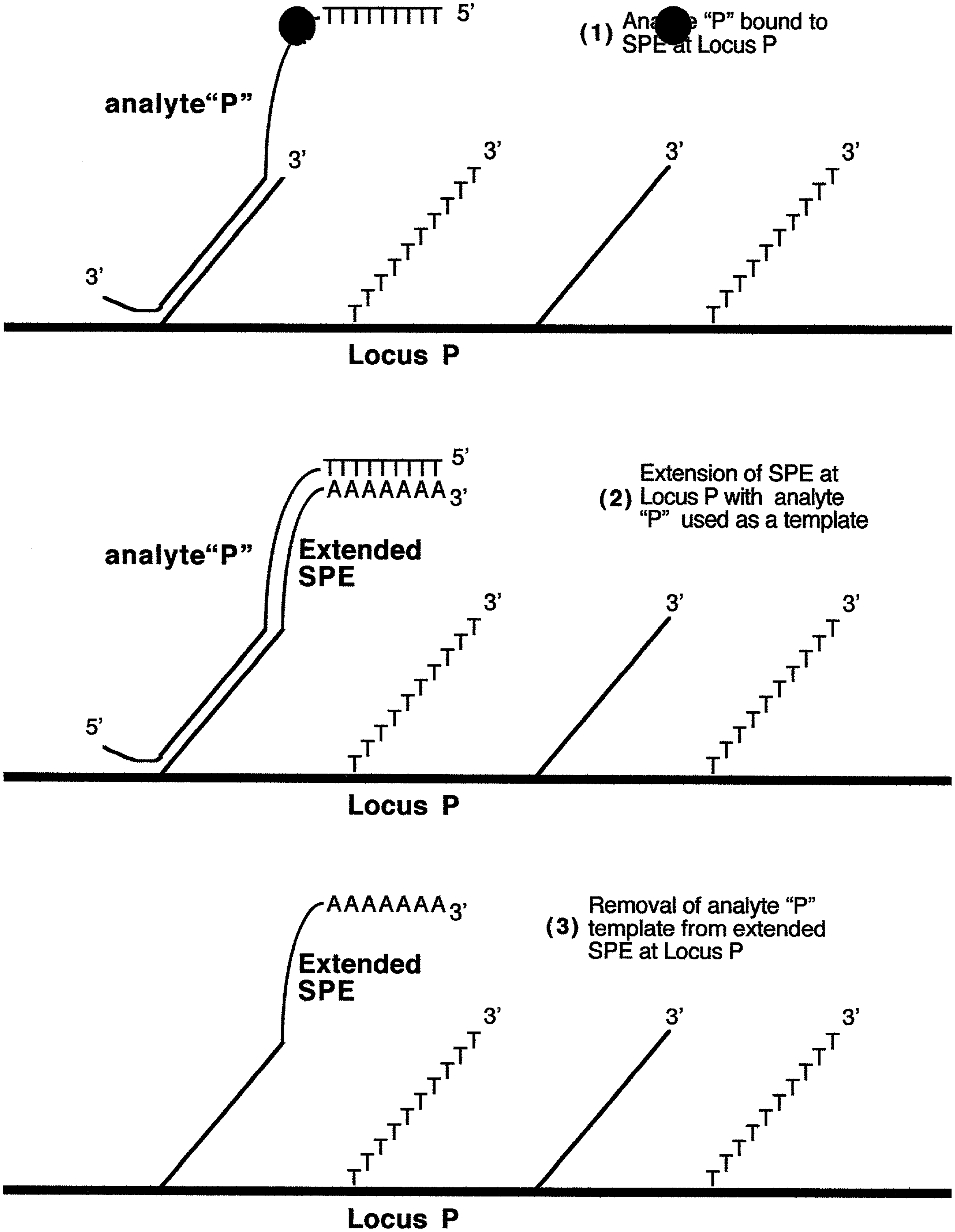


Figure 17

Extension of an SPE

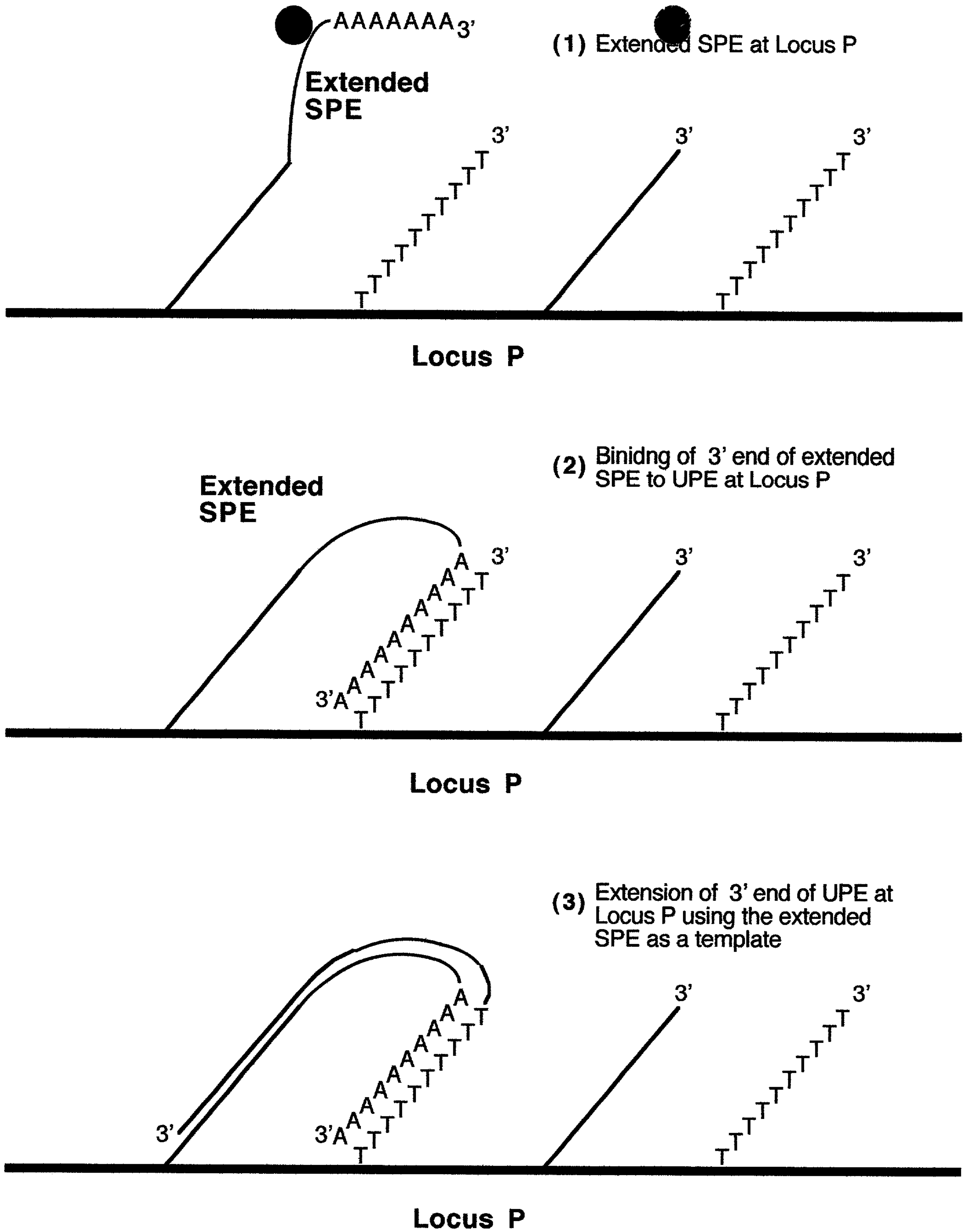


Figure 18

Binding of a UPE to an extended SPE followed by extension of the UPE

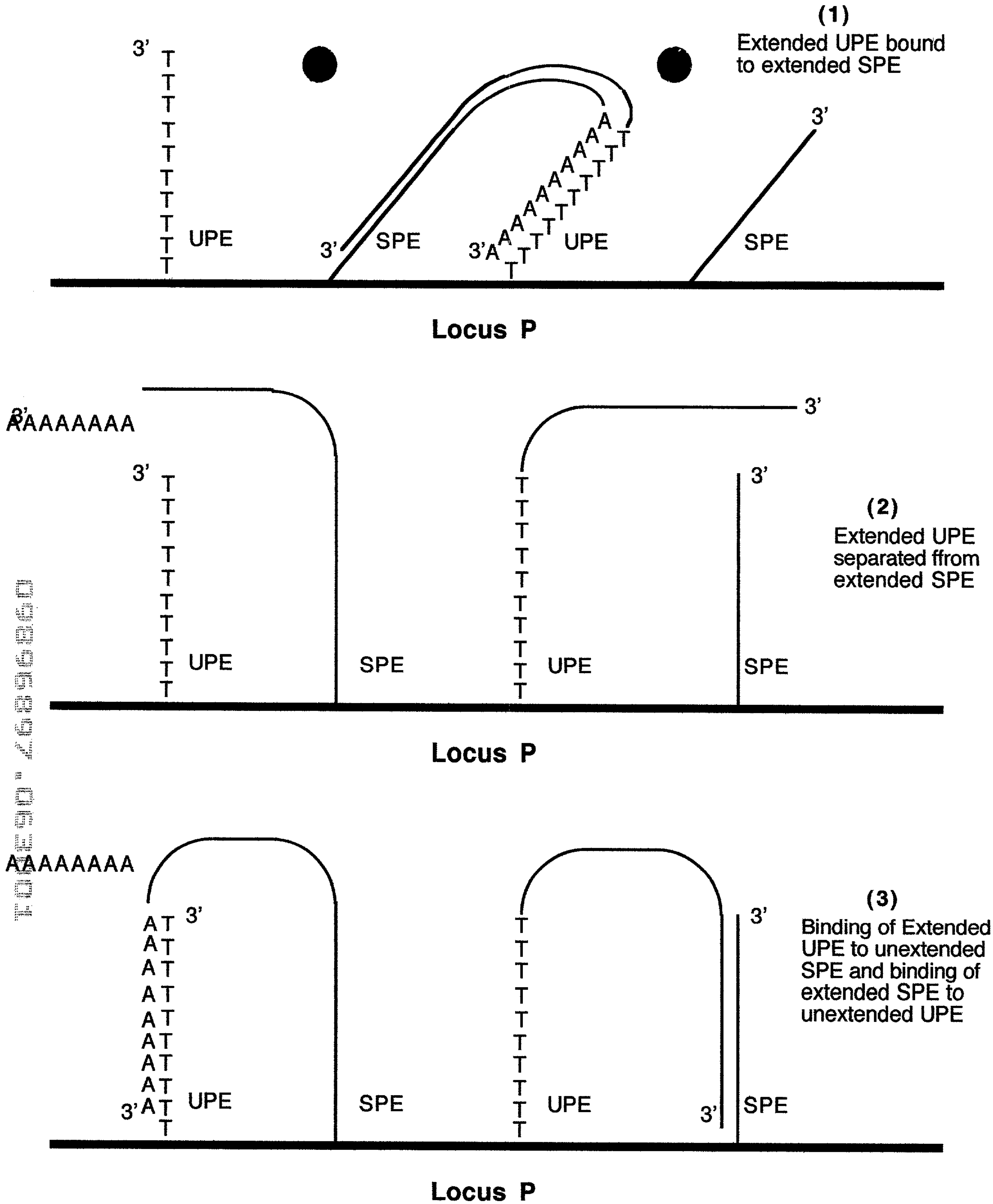


Figure 19

Binding of extended SPE's and UPE's to un-extended SPE's and UPE's

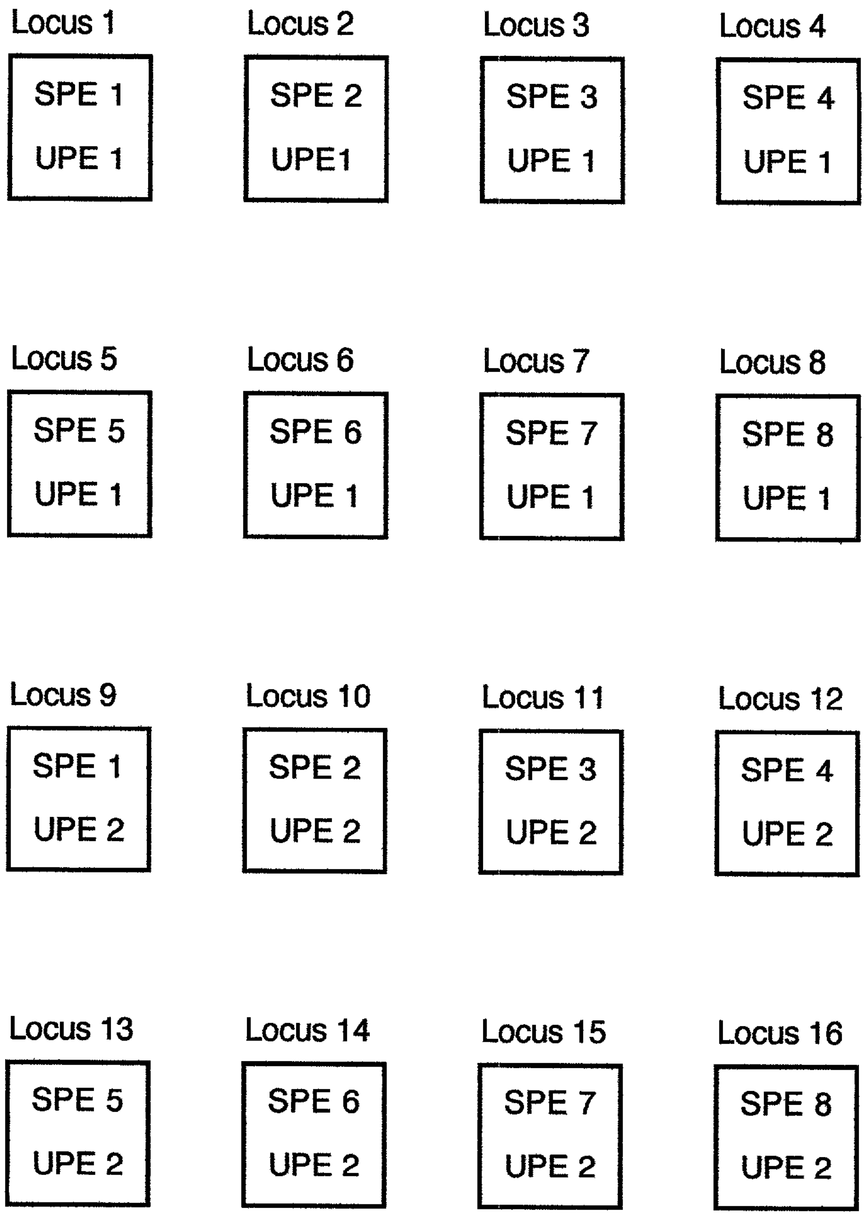


Figure 20

Amplification Array for Comparative Analysis

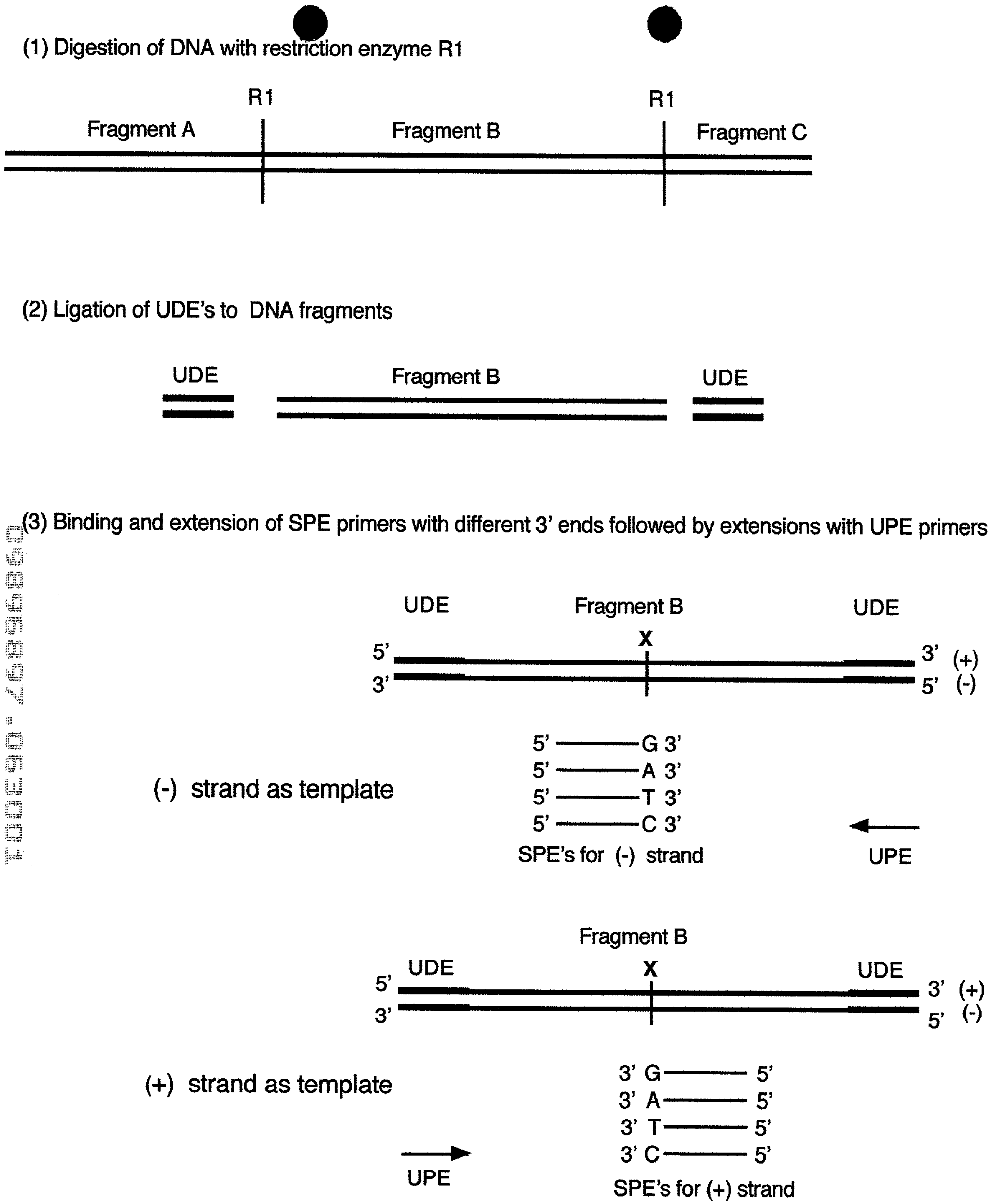


Figure 21

Use of an array with SPE's and UPE's for SNP analysis

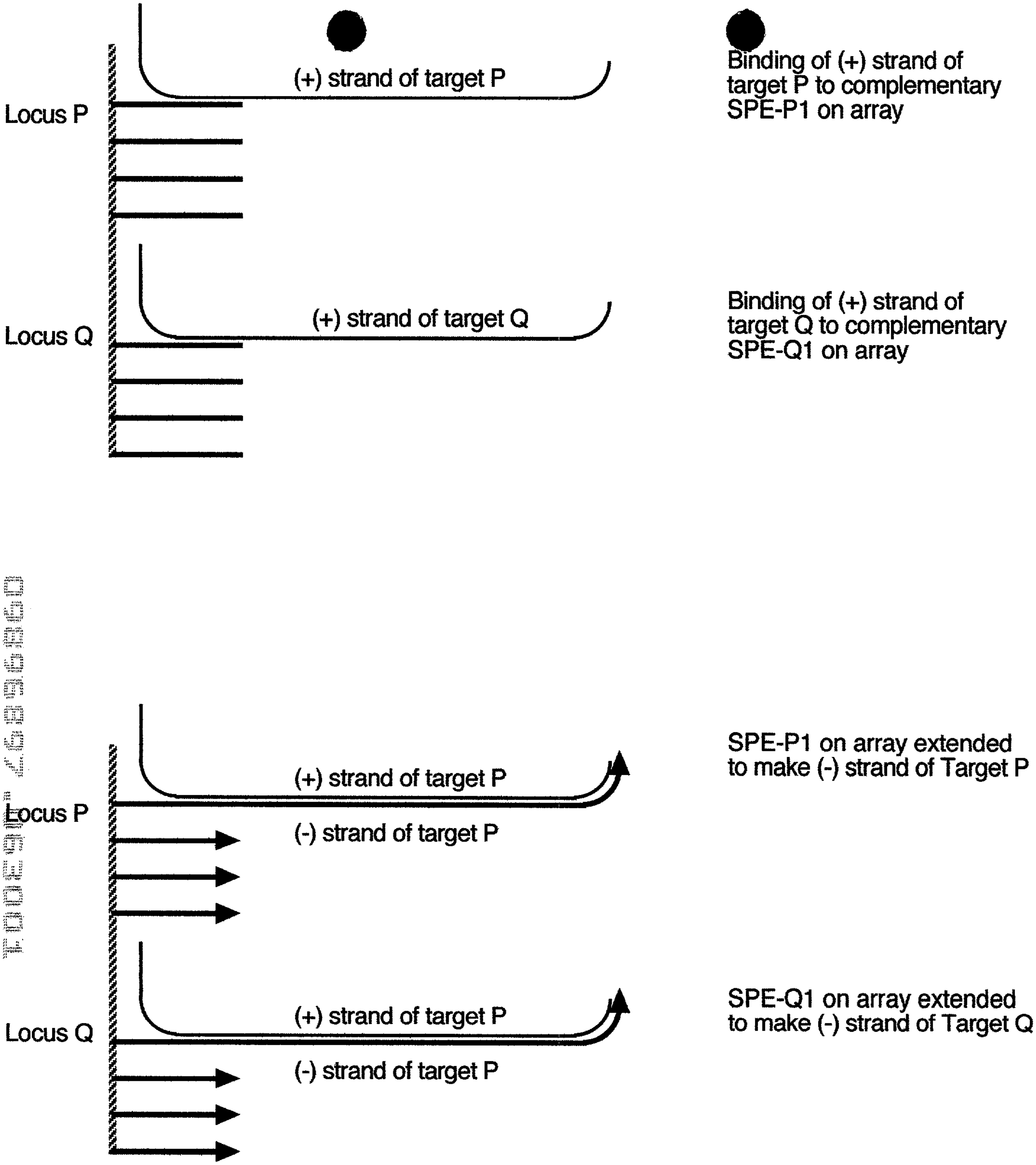

Locus P
(+) strand of target P
Binding of (+) strand of target P to complementary SPE-P1 on array
Locus Q
(+) strand of target Q
Binding of (+) strand of target Q to complementary SPE-Q1 on array
Locus P
(+) strand of target P
(-) strand of target P
SPE-P1 on array extended to make (-) strand of Target P
Locus Q
(+) strand of target P
(-) strand of target P
SPE-Q1 on array extended to make (-) strand of Target Q

Figure 22

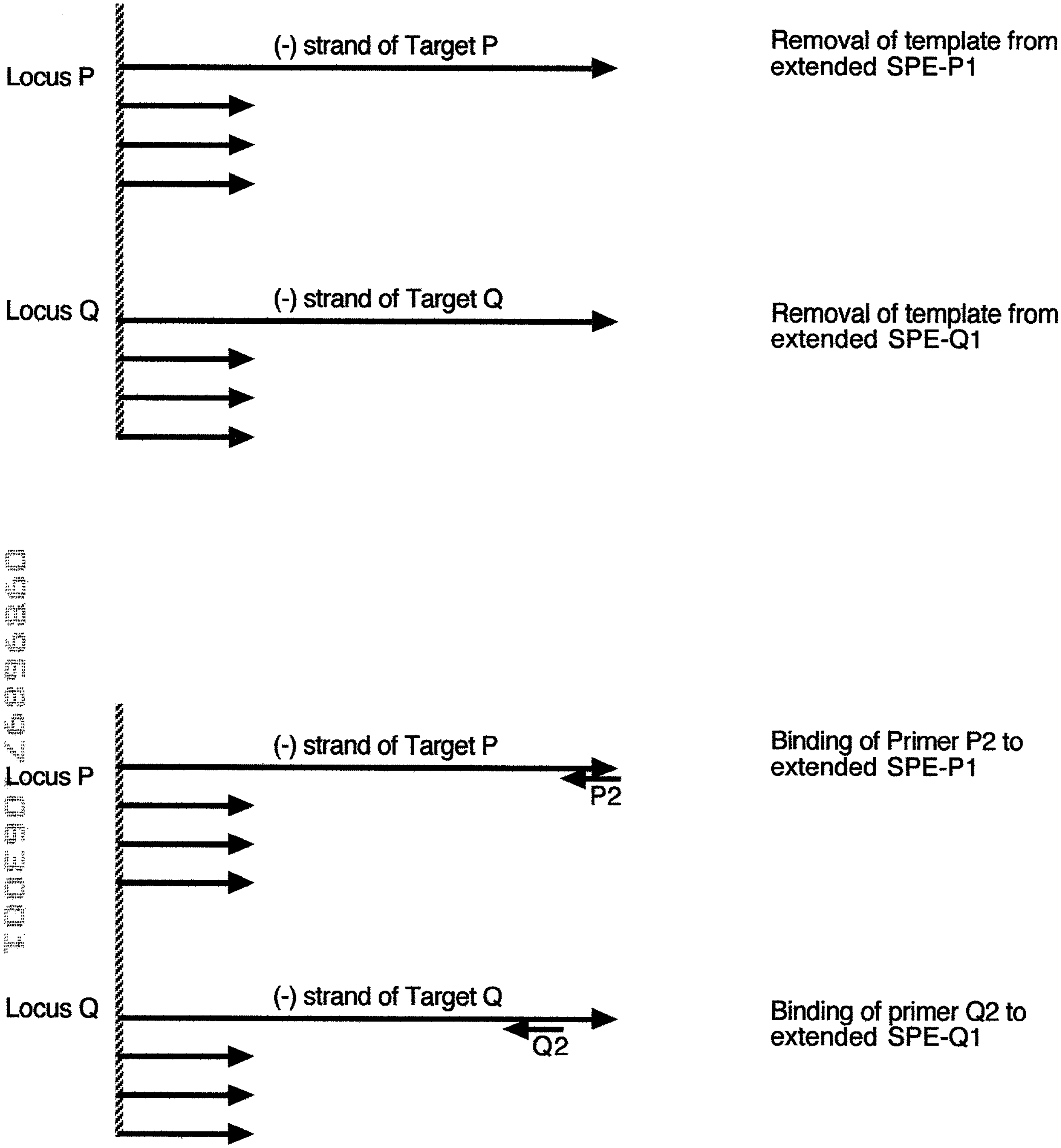
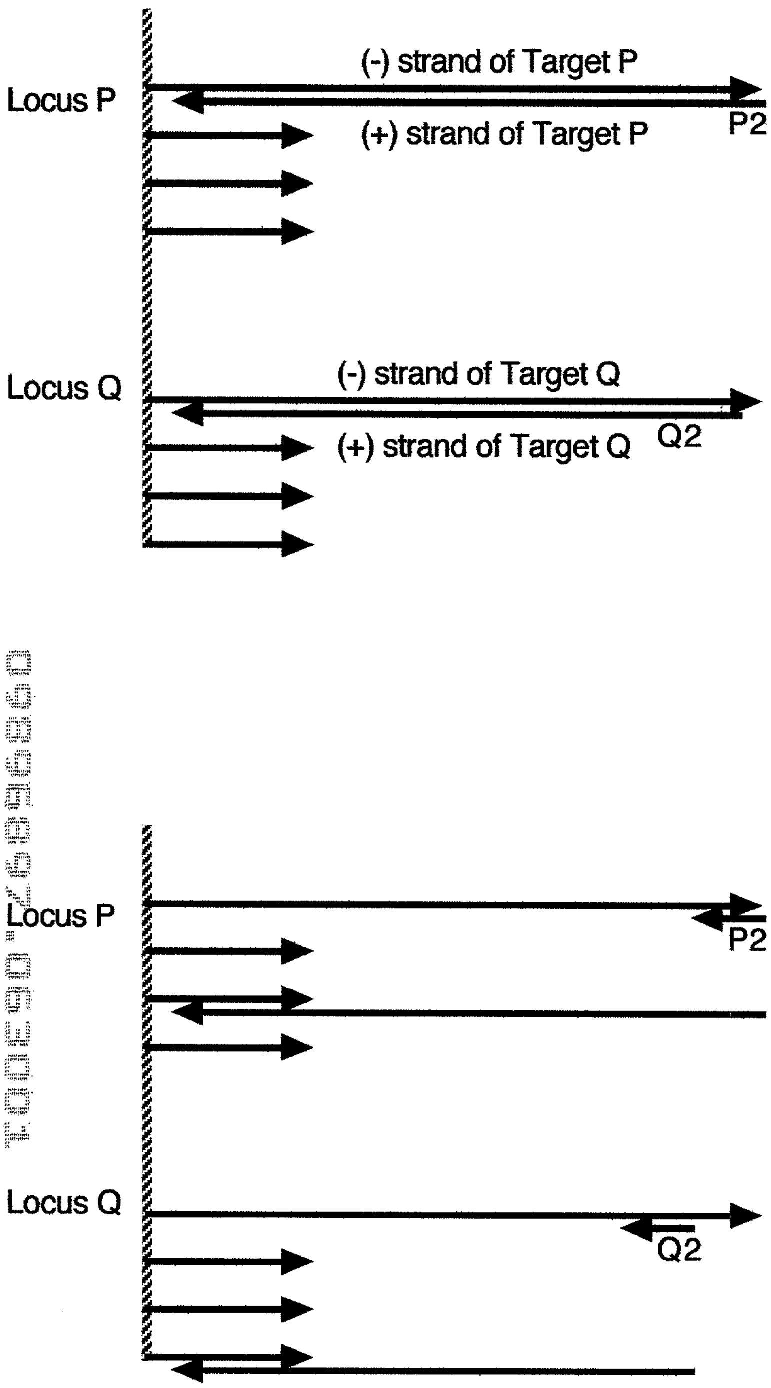

Locus P
(-) strand of Target P
Removal of template from extended SPE-P1
Locus Q
(-) strand of Target Q
Removal of template from extended SPE-Q1
Locus P
(-) strand of Target P
P2
Binding of Primer P2 to extended SPE-P1
Locus Q
(-) strand of Target Q
Q2
Binding of primer Q2 to extended SPE-Q1

Figure 23



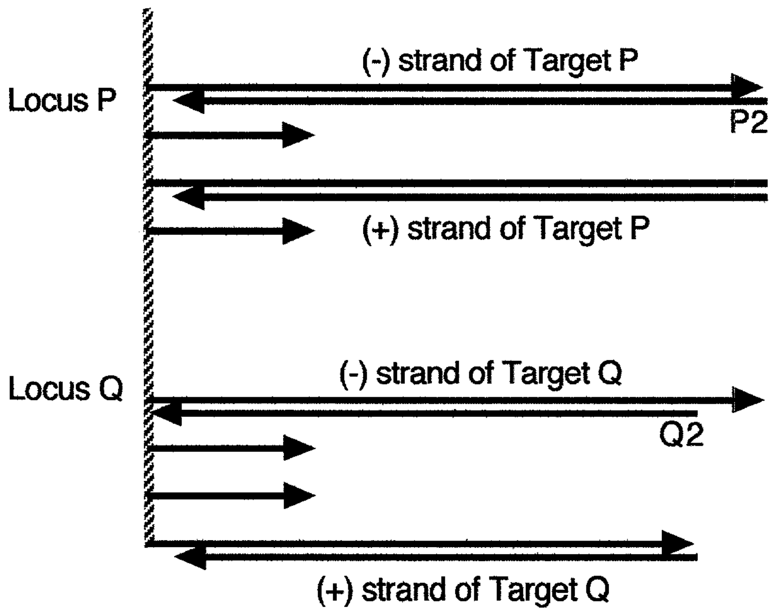
Extension of primer P2 by using extended SPE-P1 as a template

Extension of primer Q2 by using extended SPE-Q1 as a template

Denaturation followed by annealing of primer P2 to extended SPE-P1 and hybridization of extended P2 to un-extended SPE-P1

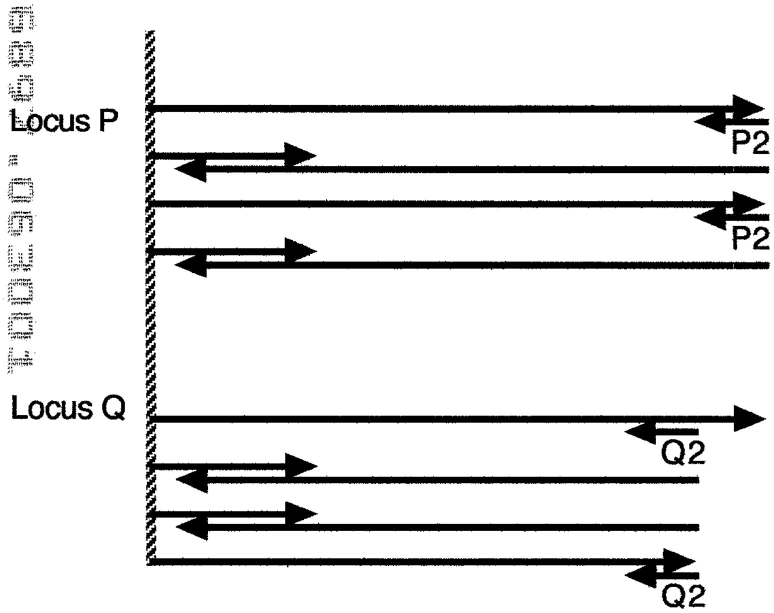
Denaturation followed by annealing of primer Q2 to extended SPE-Q1 and hybridization of extended Q2 to un-extended SPE-Q1

Figure 24



Extension of primer P2 and SPE-P1

Extension of primer Q2 and SPE-Q1



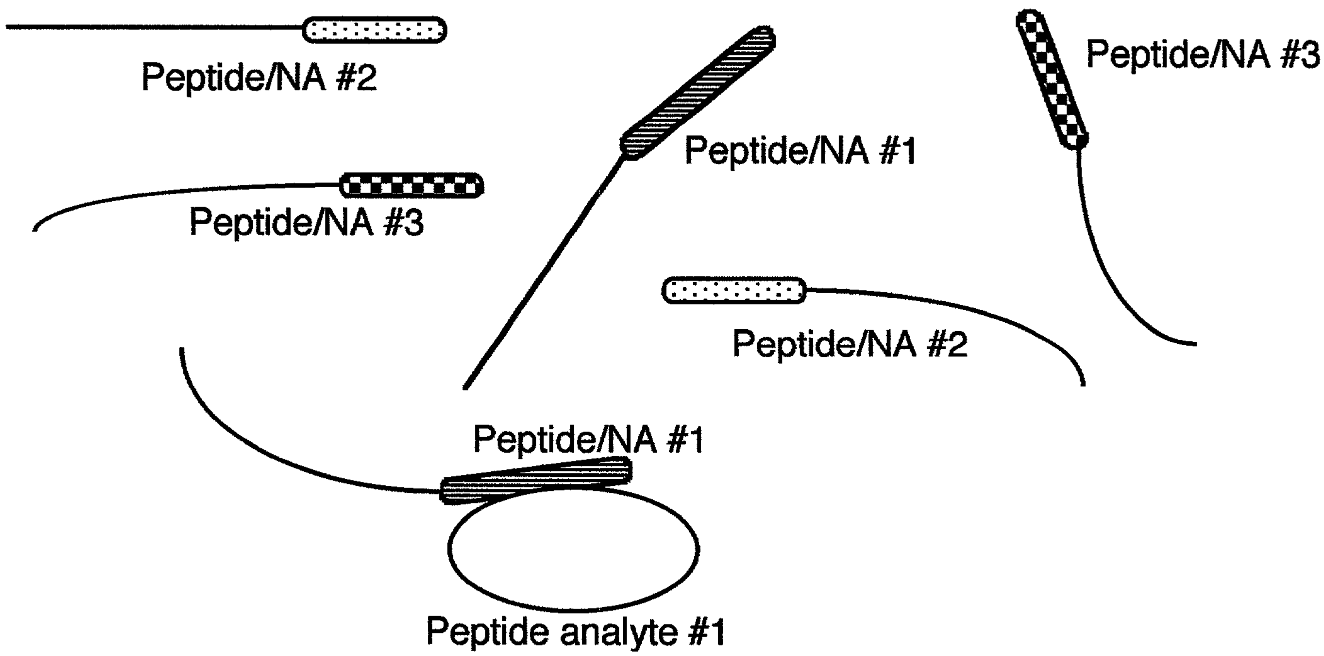
Denaturation followed by annealing of primer P2's to extended SPE-P1's and hybridization of extended P2's to un-extended SPE-P1's

Denaturation followed by annealing of primer Q2's to extended SPE-Q1's and hybridization of extended Q2's to un-extended SPE-Q1's

Figure 25

A) Mixture of a Library of Peptide analytes with a Library of Peptide/NAs

B) Binding of Peptide analyte #1 to Peptide/NA #1



C) Binding of Peptide/NAs to matrix through complementary sequences

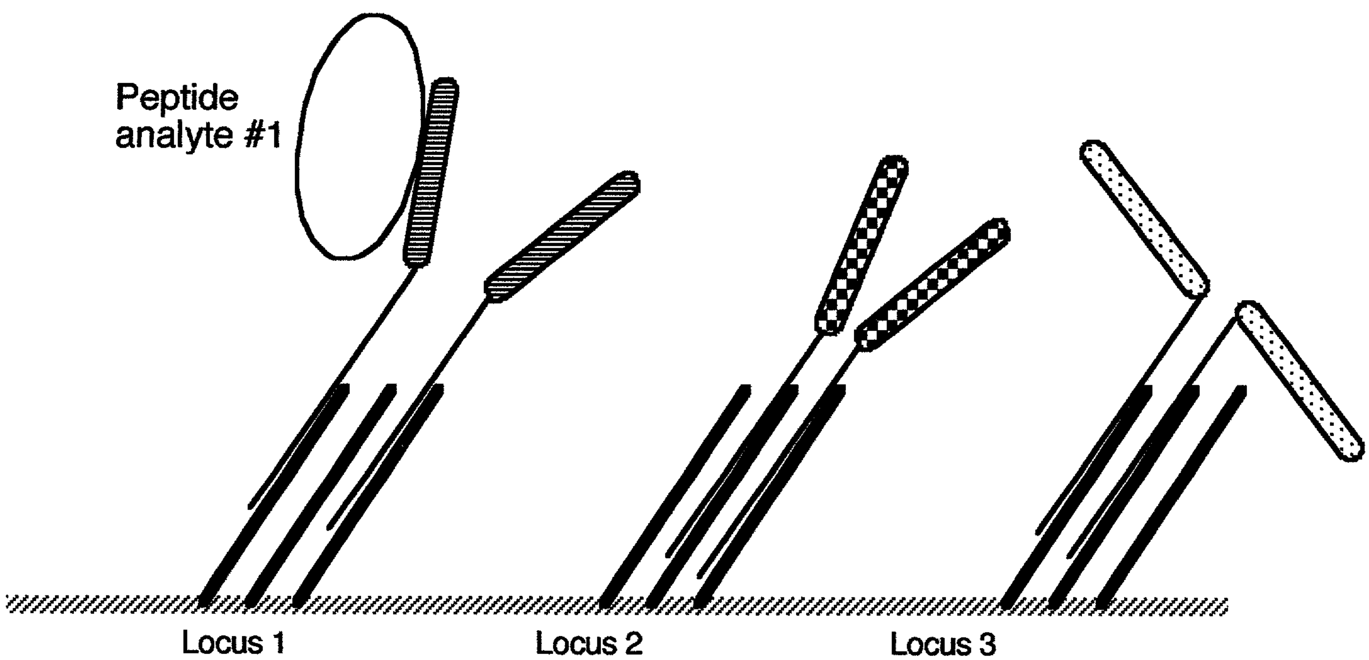
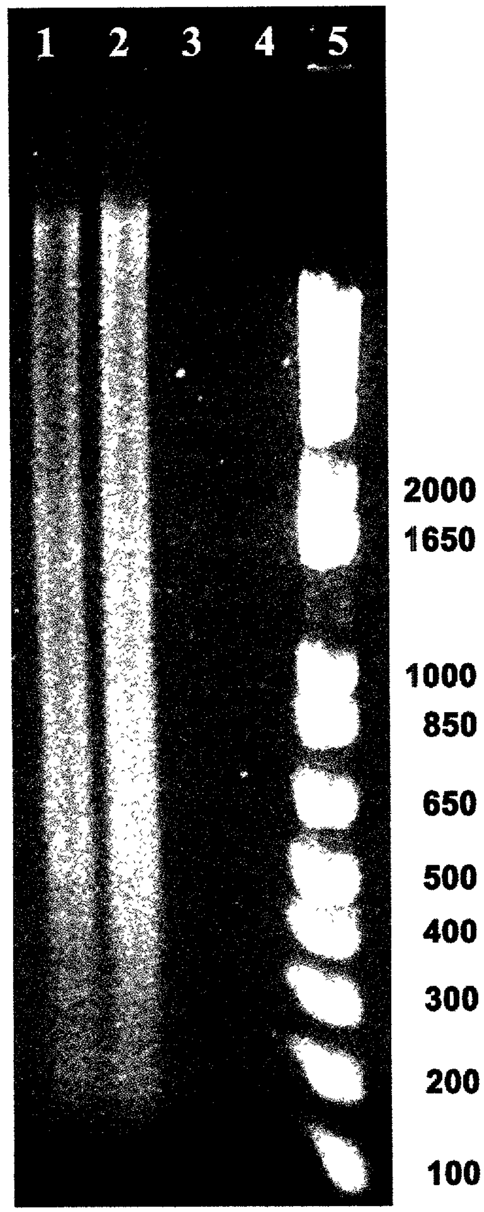


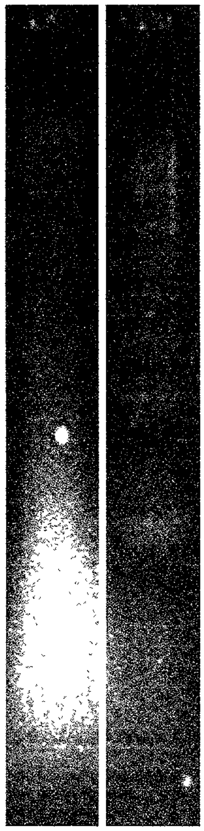
Figure 26



1. "+" RT
2. "+" RT
3. "-" RT
4. "-" RT
5. 1 kb PLUS DNA Ladder

Figure 27

1 2



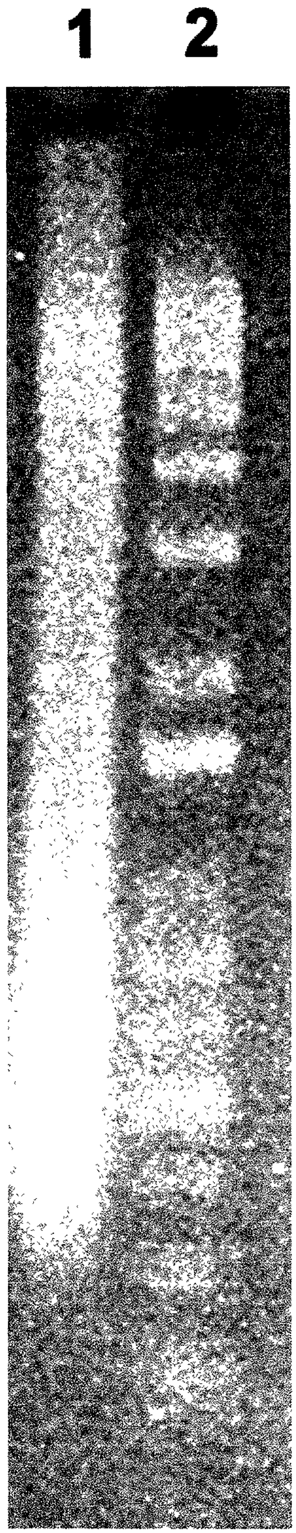
1. Transcription product

2. 1kb PLUS DNA ladder

2000 bp

1650 bp

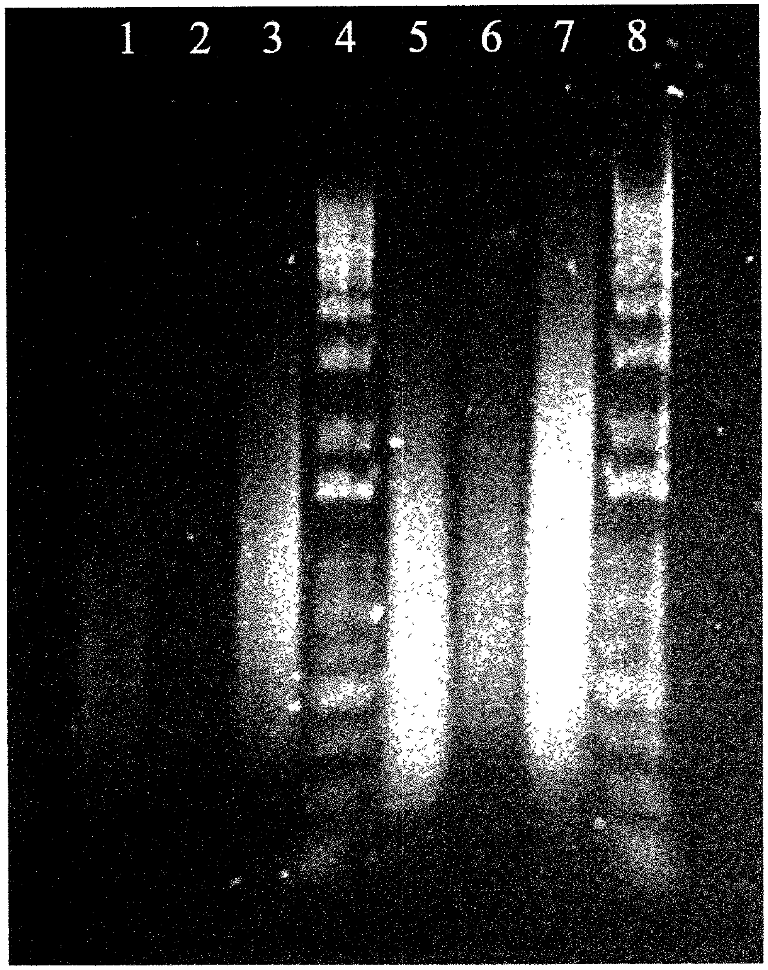
Figure 28



1. Transcription Product

2. 1 kb PLUS DNA Ladder

Figure 29



1. Random primers - 2 µl

2. T7-C9 primers without TdT tailing - 2 µl

3. T7-C9 primers after TdT tailing - 2 µl

4. 1 kb PLUS DNA Ladder

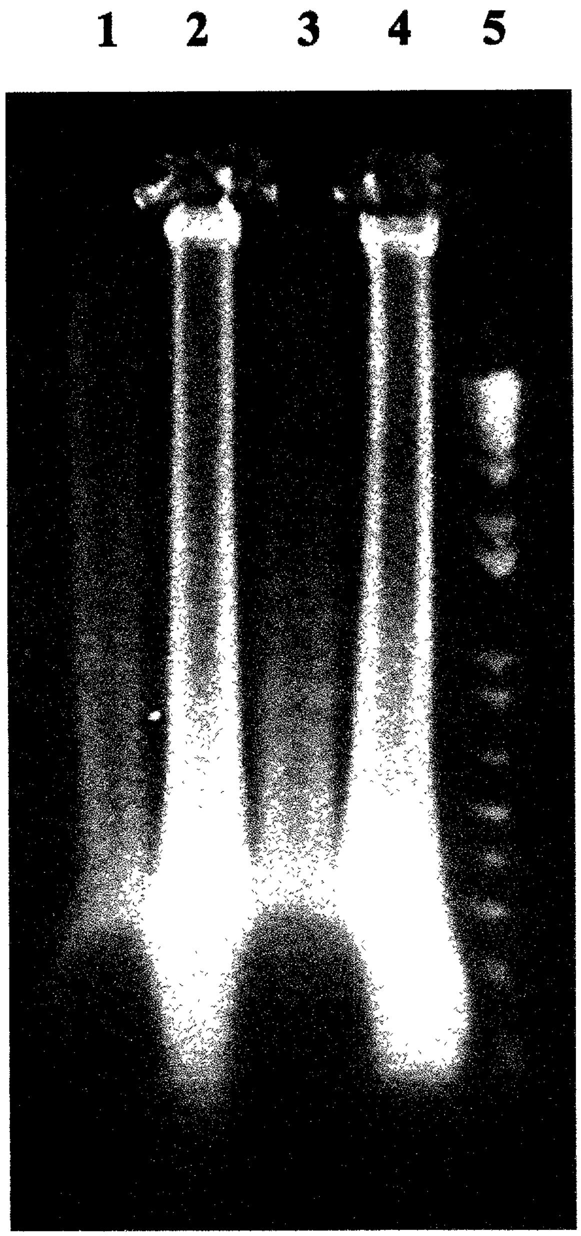
5. Random primers - 10 µl

6. T7-C9 primers without TdT tailing - 10 µl

7. T7-C9 primers after TdT tailing - 10 µl

8. 1 kb PLUS DNA Ladder

Figure 30



1. Taq pol. 1 cycle

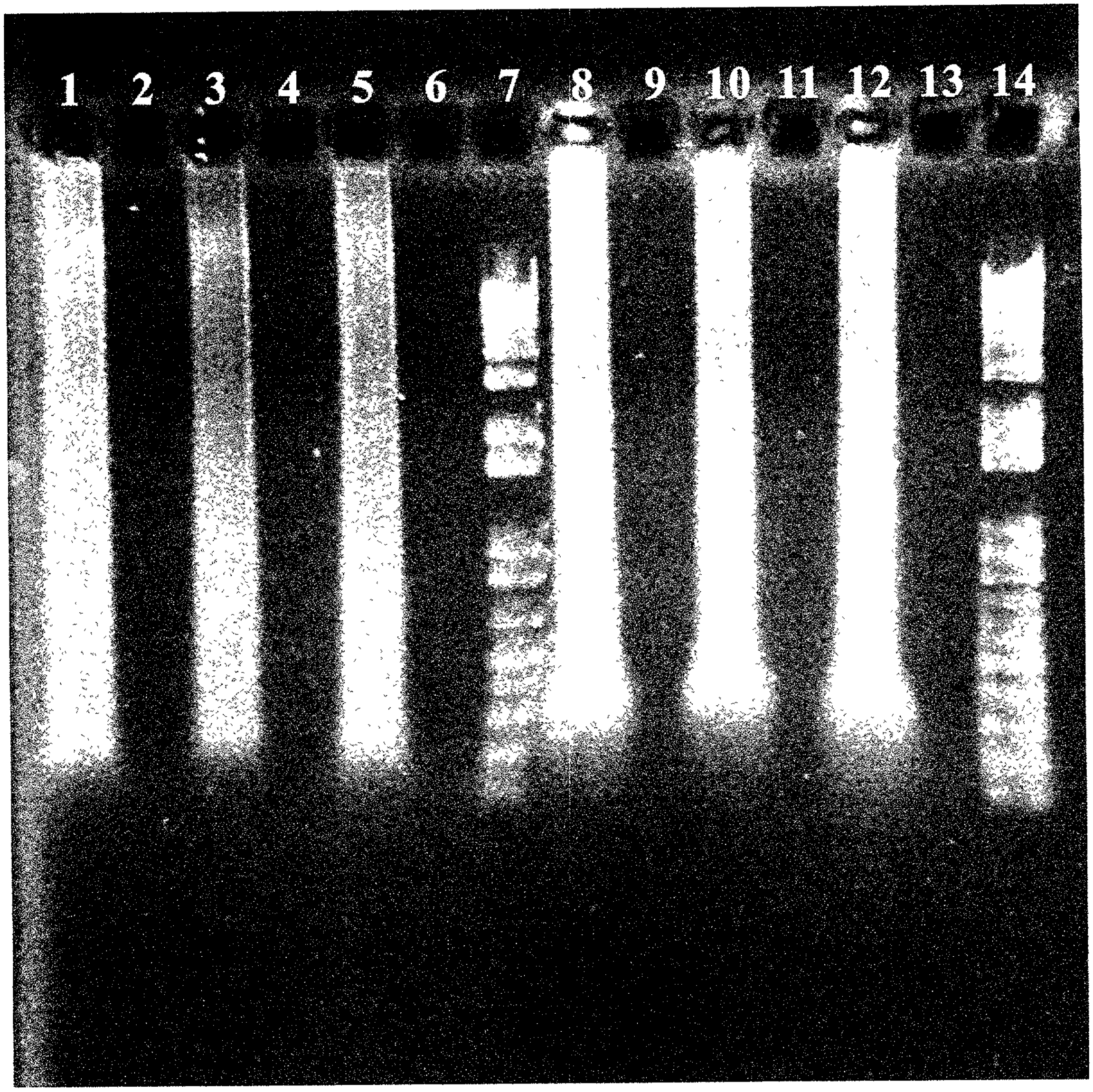
2. Taq pol. 5 cycles

3. Tth pol. 1 cycle

4. Tth pol. 5 cycles

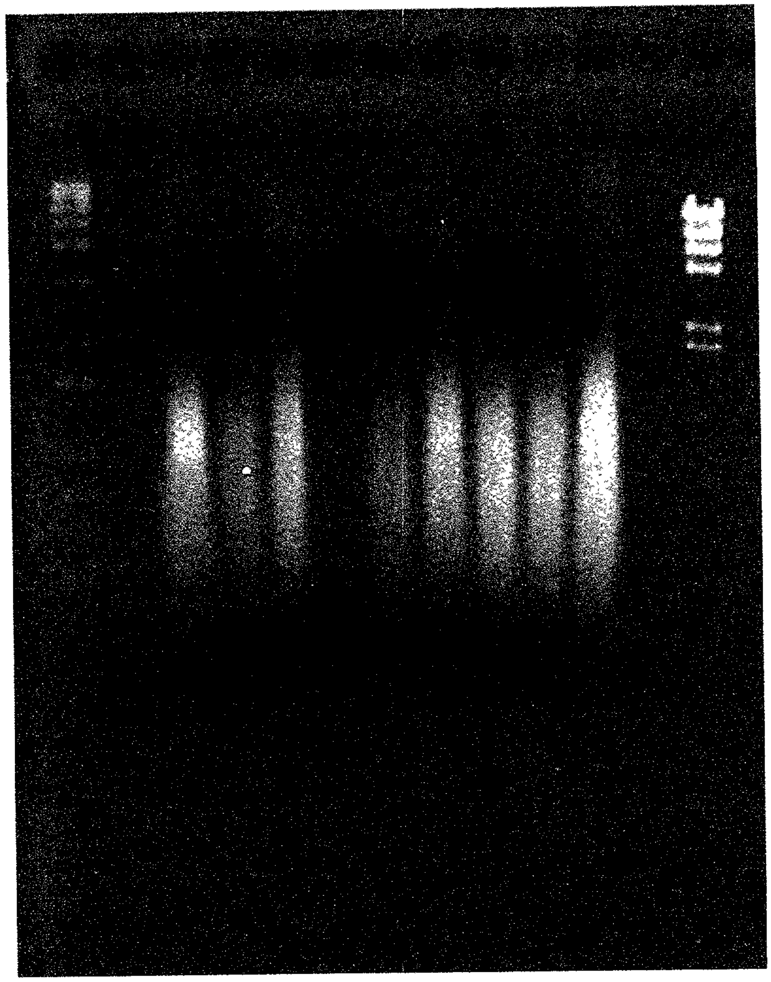
5. 1 kb PLUS DNA Ladder

Figure 31



1. Sample 1 - 4 µl transcription product
2. Sample 1 - 1 µl DNA template
3. Sample 2 - 4 µl transcription product
4. Sample 2 - 1 µl DNA template
5. Sample 3 - 4 µl transcription product
6. Sample 3 - 1 µl DNA template
7. 1 kb PLUS DNA Ladder
8. Sample 1 - 10 µl transcription product
9. Sample 1 - 2.5 µl DNA template
10. Sample 2 - 10 µl transcription product
11. Sample 2 - 2.5 µl DNA template
12. Sample 3 - 10 µl transcription product
13. Sample 3 - 2.5 µl DNA template
14. 1 kb PLUS DNA Ladder

Figure 32



1. 1 kb PLUS DNA Ladder
2. - - - -
3. Superscript II (Life Technologies)
4. M-MuLV (Life Technologies)
5. M-MuLV (New England Biolabs)
6. Enhanced AMV (Sigma)
7. AMV (Life Technologies)
8. AMV (Sigma)
9. Omniscript (Qiagen)
10. displayTHERMO-RT (Display systems Biotech)
11. Powescript (Clontech)
12. - - - -
13. λ - Hind III marker

Figure 33